QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD (Q)SAR Toolbox v.4.4.1

An example illustrating RAAF scenario 6 and related assessment elements

Outlook

- **Background**
- Keywords
- Objectives
- Specific Aims
- Read Across Assessment Framework (RAAF)
- The exercise
- Workflow

Background

- This is a step-by-step presentation designed to take the Toolbox user through the workflow of a data gap filling exercise and assessing of the outcome whether the read-across prediction is scientifically acceptable or not;
- The read-across prediction will be justified by fulfilling all information requirements according to the Read Across Assessment Framework (RAAF).

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Keywords

TARGET CHEMICAL - chemical of interest.

MODULE – a Toolbox module is a section dedicated to specific actions and options (e.g. Profiling).

WORKFLOW – the use, in combination, of the different modules (e.g. prediction workflow: from input to report).

PROFILER - algorithm (rule set) for the identification of specific features of the chemicals. Several types of profilers are available, such as structural (e.g. Organic functional groups), mechanistic (e.g. Protein binding by OECD) and endpoint-specific (e.g. in vitro in vitro mutagenicity (Ames test) alerts by ISS) profilers.

ALERT - the profilers consist of sets of rules or alerts. Each of the rules consists of a set of queries. The queries could be related to the chemical structure, physicochemical properties, experimental data, comparison with the target or list with substances and external queries from other predefined profilers (reference queries).

CATEGORY – "group" of substances sharing same characteristics (e.g. the same functional groups or mode of action). In a typical Toolbox workflow, it consists of the target chemical and its analogues gathered according to the selected profilers.

ENDPOINT TREE – Endpoints are structured in a branched scheme, from a broader level (Phys-Chem properties, Environmental Fate and transport, Ecotoxicology, Human health hazard) to a more detailed one (e.g. EC3 in LLNA test under Human health hazard-Skin sensitization).

DATA MATRIX – Table reporting the chemical(s) and data (experimental results, profilers outcomes, predictions). Each chemical is in a different column and each data in a different row.

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Objectives

This presentation demonstrates a number of functionalities of the Toolbox:

- Define target endpoint;
- Relevancy of profiles and data availability;
- Calculation of alert performance (AP) accounting for metabolism;
- Searching of analogues accounting for metabolism;
- Category consistency check;
- Selection of RAAF scenario;
- Filling in the report sections related to each read-across assessment element.

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Specific Aims

- To familiarize the user with the Read Across Assessment Framework (RAAF) and more specifically with Scenario 6;
- To introduce to the user the read-across assessment elements;
- To introduce to the user the report basket;
- To provide sufficient information allowing a scientific assessment of the outcome;
- To explain to the Toolbox user the rationale behind each step of the exercise.

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Read Across Assessment Framework (RAAF)

Overview

- RAAF has been developed by ECHA as internal tool providing a framework for a consistent and structured assessment of grouping and read-across approaches under REACH.
- The outcome of the assessment is a conclusion on whether the read-across is scientifically acceptable or not
- The RAAF defines different scenarios for different read-across approaches
- Each scenario is associated with particular aspects (assessment elements, AEs) that are deemed crucial to the assessment
- Total six scenarios are available: two for analogue approach and four for category approach

Read Across Assessment Framework (RAAF)

Criteria for the different RAAF scenarios

SCENARIO	APPROACH	READ-ACROSS HYPOTHESIS BASED ON	QUANTITATIVE VARIATIONS
1	Analogue	(Bio)transformation to common compound(s)	Property of the target substance predicted to be quantitatively equal to those of the source substance or prediction based on a worst-case approach.
2	Analogue	Different compounds have qualitatively similar properties	Properties of the target substance predicted to be quantitatively equal to those of the source substance or prediction based on a worst-case approach.
3	Category	(Bio)transformation to common compound(s)	Variations in the properties observed among source substances. Prediction based on a regular pattern or on a worst-case approach.
4	Category	Different compounds have qualitatively similar properties	Variations in the properties observed among source substances. Prediction based on a regular pattern or on a worst-case approach.
5	Category	(Bio)transformation to common compound(s)	No relevant variations in properties observed among source substances and the same strength predicted for the target substance.
6	Category	Different compounds have qualitatively similar properties	No relevant variations in properties observed among source substances and the same strength predicted for the target substance

*Read-Across Assessment Framework (RAAF) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

Read Across Assessment Framework (RAAF)

Selection of RAAF scenario

1. Distinguish whether analogue or category approach is decided based on number (N) of analogues*:
   a) N of analogues ≤ 3 is Analogue approach (scenario 1-2)
   b) N of analogues > 3 is Category approach (scenario 3-6)
2. To identify the basis of the read across hypothesis
   a) (Bio)transformation to common compound(s) – the read-across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed
   b) Different compounds have the same type of effect(s) – the read-across hypothesis is that the organism is not exposed to common compounds but rather, as a result of similarity, that different compounds have similar (eco)toxicological and fate properties. These compounds may be the **source** and **target substances themselves** or **one or more of their (bio)transformation products**.
3. For a category approach (scenario 3-6) there is a need to take further account whether or not quantitative variations in the properties are observed among the category members:
   a) There is quantitative variation in the (eco) toxicity when it is more than 1 log units** (scenario 3 and 4)
   b) Quantitative variation is not expected in the (eco) toxicity when it is less or equal to 1 log unit (scenario 5-6)

*** The threshold for number of analogues which distinguishes analogue from category approach is proposed by LMC**
****The quantitative variation in the (eco)toxicity of 1 log unit is proposed by LMC due to empirically observations.**

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The Exercise

- In this exercise we will predict a *Chromosomal aberration (CA)* of 3,5-Dichlorophenol [CAS# 591-35-5], which will be the "target" chemical.
- We will preliminary define the target endpoint;
- The category will be defined by DNA binding mechanism accounting for *in vitro* rat liver metabolism;
- The read-across approach will be used for the prediction. The read-across will be based on category approach expressed as common underlying mechanism for metabolites of source and target substances;
- Read-across assessment elements will be included to the report
- Examples for the possible content of each of AEs will be provided

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Workflow

The Toolbox has six modules which are used in a sequential workflow:

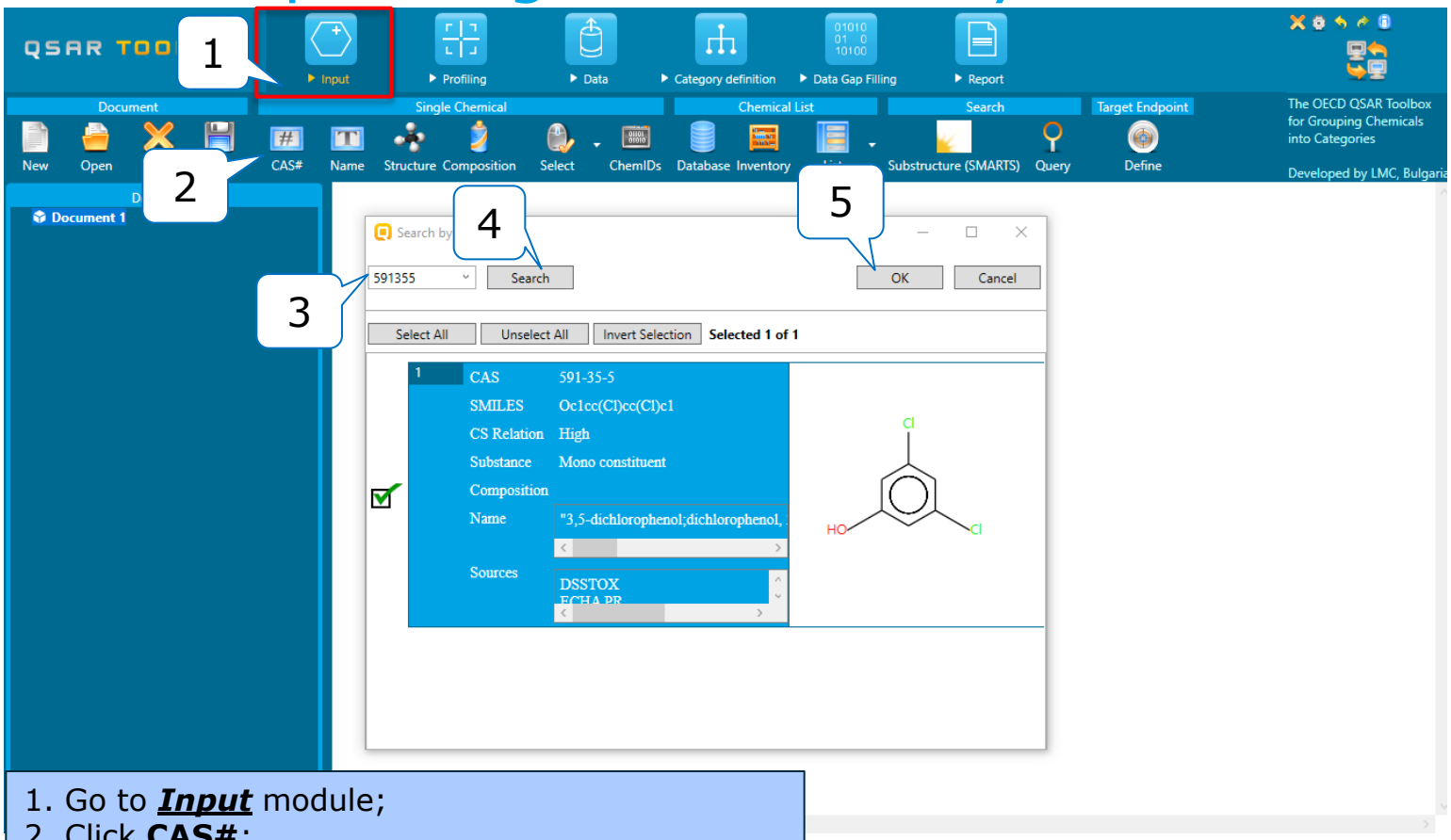
- Input
- Profiling
- Data
- Category Definition
- Data Gap Filling
- Report

Input
Overview

- This module provides the user with several means of entering the chemical of interest or the target chemical.
- Since all subsequent functions are based on chemical structure, the goal here is to make sure the molecular structure assigned to the target chemical is the correct one.

Input

Input target chemical by CAS#

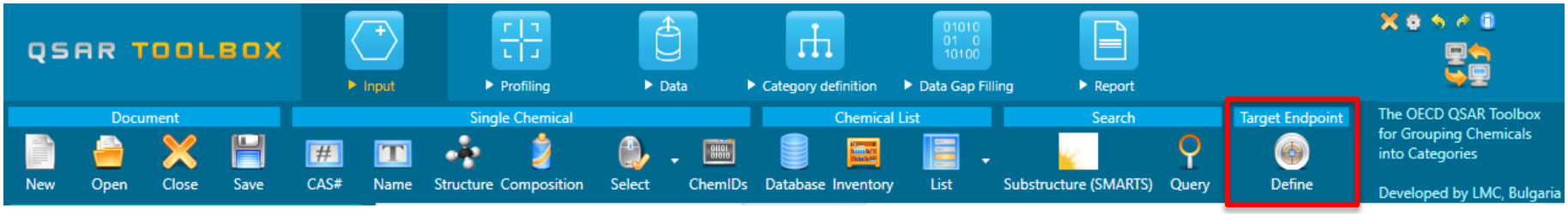


1. Go to ***Input*** module;
2. Click **CAS#**;
3. Enter the **CAS# 591-35-5** in the blank field;
4. Click ***Search;***
5. When the structure appears click ***OK.***

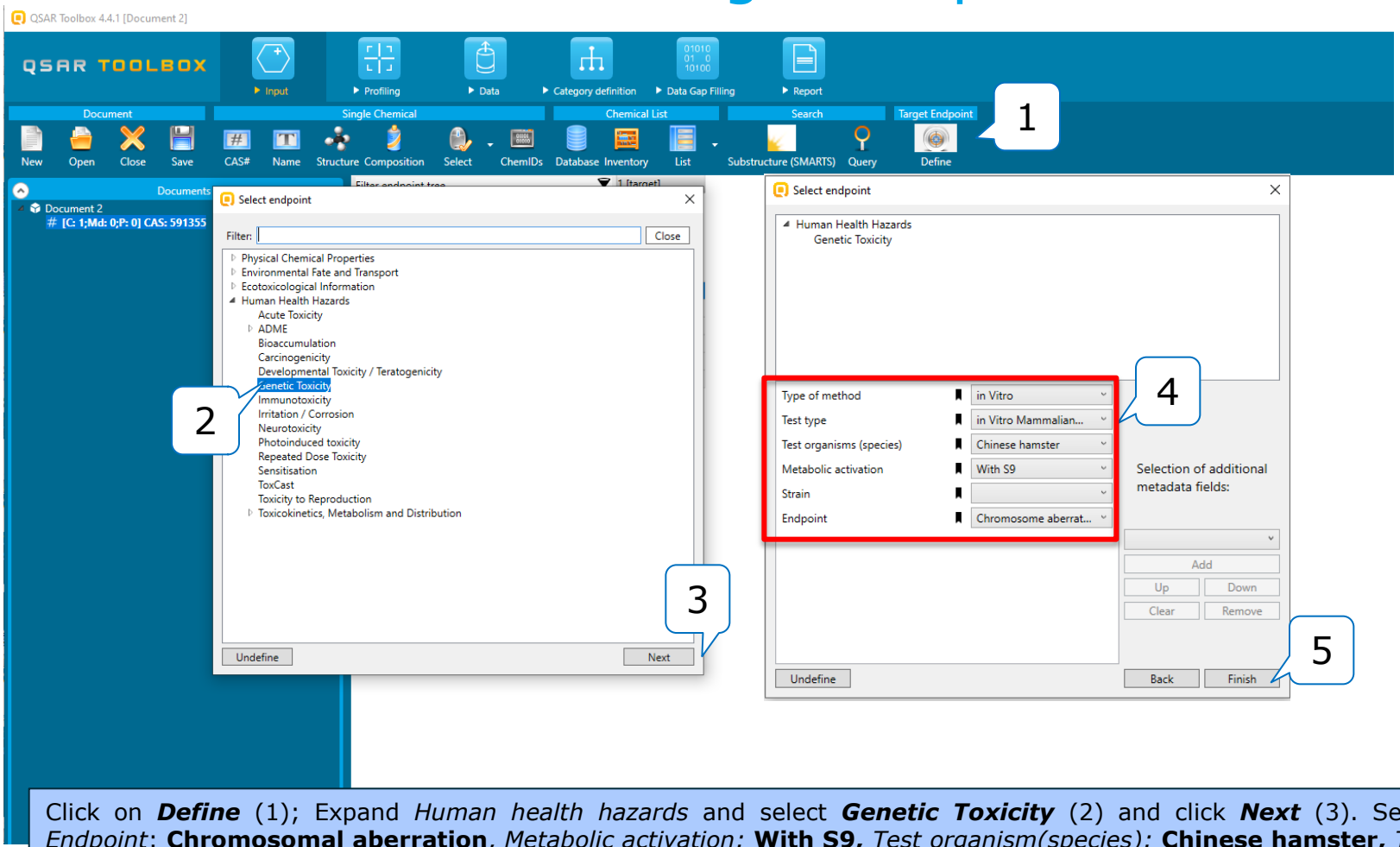
Input
Define target endpoint

Defining of the endpoint allows entering the endpoint of interest e.g. Chromosome aberration, EC3, LC50 etc., along with specific metadata information. Based on the metadata, different relevancy scores for profiles could be provided for same endpoint.

Calculation of alert performance (AP) is only possible if the target endpoint is preliminary selected.



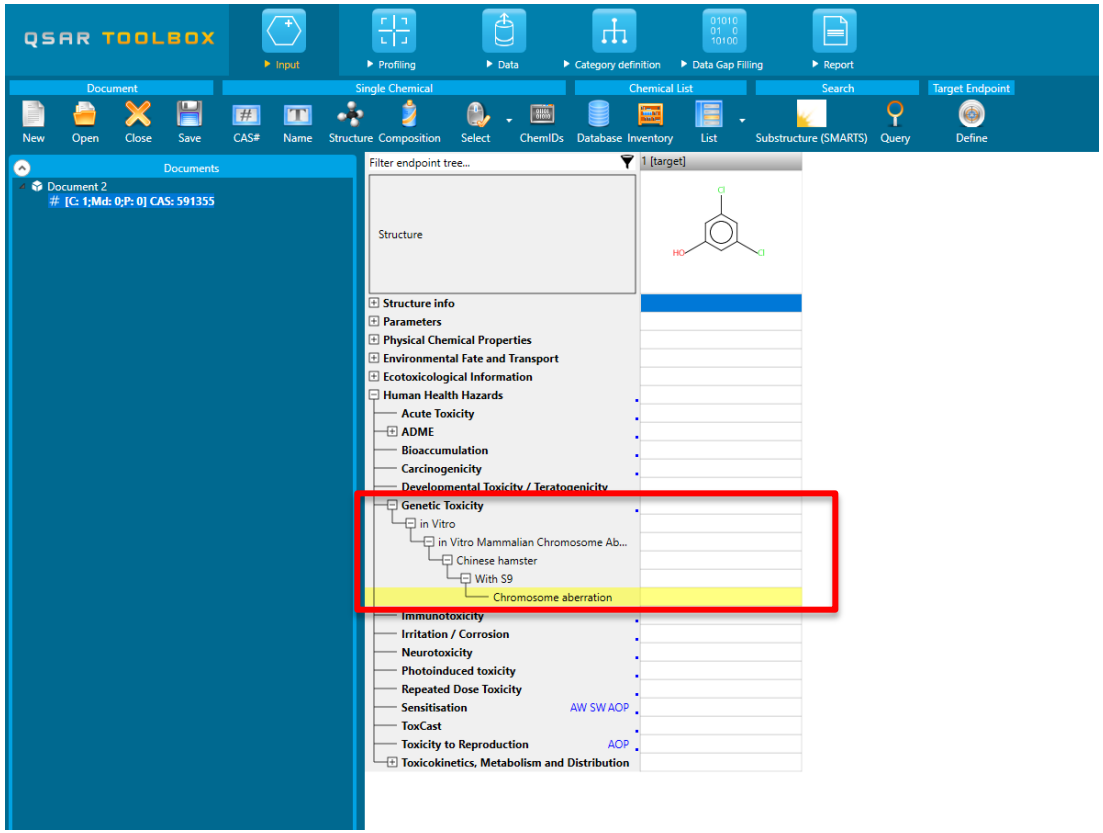
Input
Define target endpoint



Click on ***Define*** (1); Expand *Human health hazards* and select ***Genetic Toxicity*** (2) and click ***Next*** (3). Select *Endpoint*: **Chromosomal aberration**, *Metabolic activation:* **With S9,** *Test organism(species):* **Chinese hamster,** *Test type:* **In Vitro Mammalian Chromosome aberration test,** *Type of method*: **In Vitro** (4). Finally click on ***Finish*** (5).

Input
Define target endpoint

Once the endpoint is defined along with its metadata, they appear in the endpoint tree and the corresponding row of the data matrix is yellow highlighted.

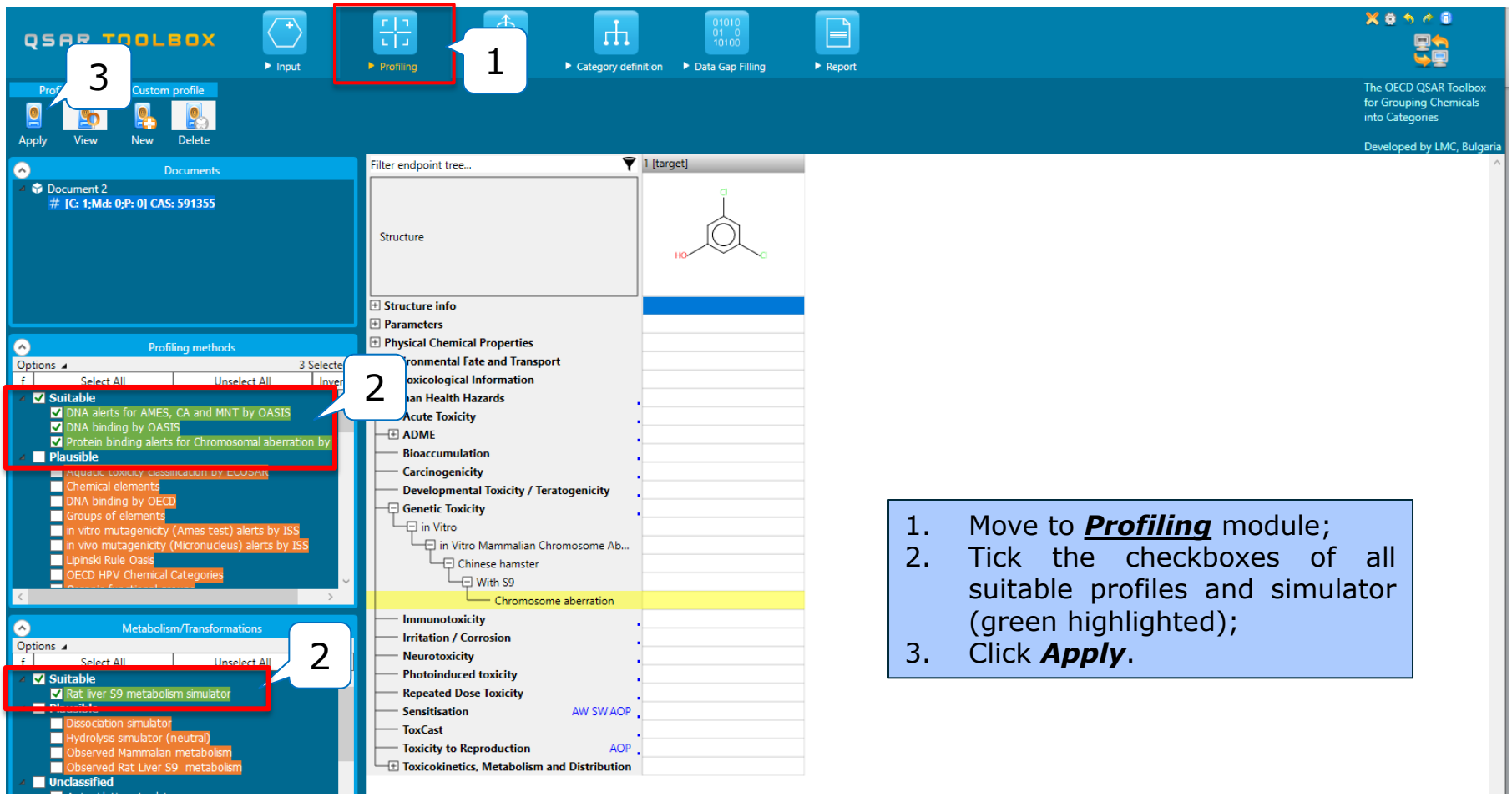


Profiling
Overview

- "Profiling" refers to the electronic process of retrieving relevant information on the target compound, other than environmental fate, ecotoxicity and toxicity data, which are stored in the Toolbox database;
- "Profiling" module contains all the knowledge in the system coded in profiling schemes (profilers);
- "Profilers" are a collection of empirical and mechanism knowledge (expertly derived) which could be used to analyse the structural properties of chemicals;
- The "profilers" identify the affiliation of the target chemical(s) to preliminary defined categories (functional groups/alerts);
- The "Profiling" module contains also observed and simulated metabolisms/transformations, which could be used in combination with the profiling schemes;
- The outcome of the profiling determines the most appropriate way to search for analogues, but they are also useful for preliminary screening or prioritization of substances;
- The "profilers" are not (Q)SARs, i.e. they are not prediction models themselves;
- Based on the "profilers' relevancy" (determined by the defined target endpoint), the most suitable once are getting colour highlighted.

Profiling

Profiling the target chemical



1. Move to ***Profiling*** module;
2. Tick the checkboxes of all suitable profiles and simulator (green highlighted);
3. Click ***Apply***.

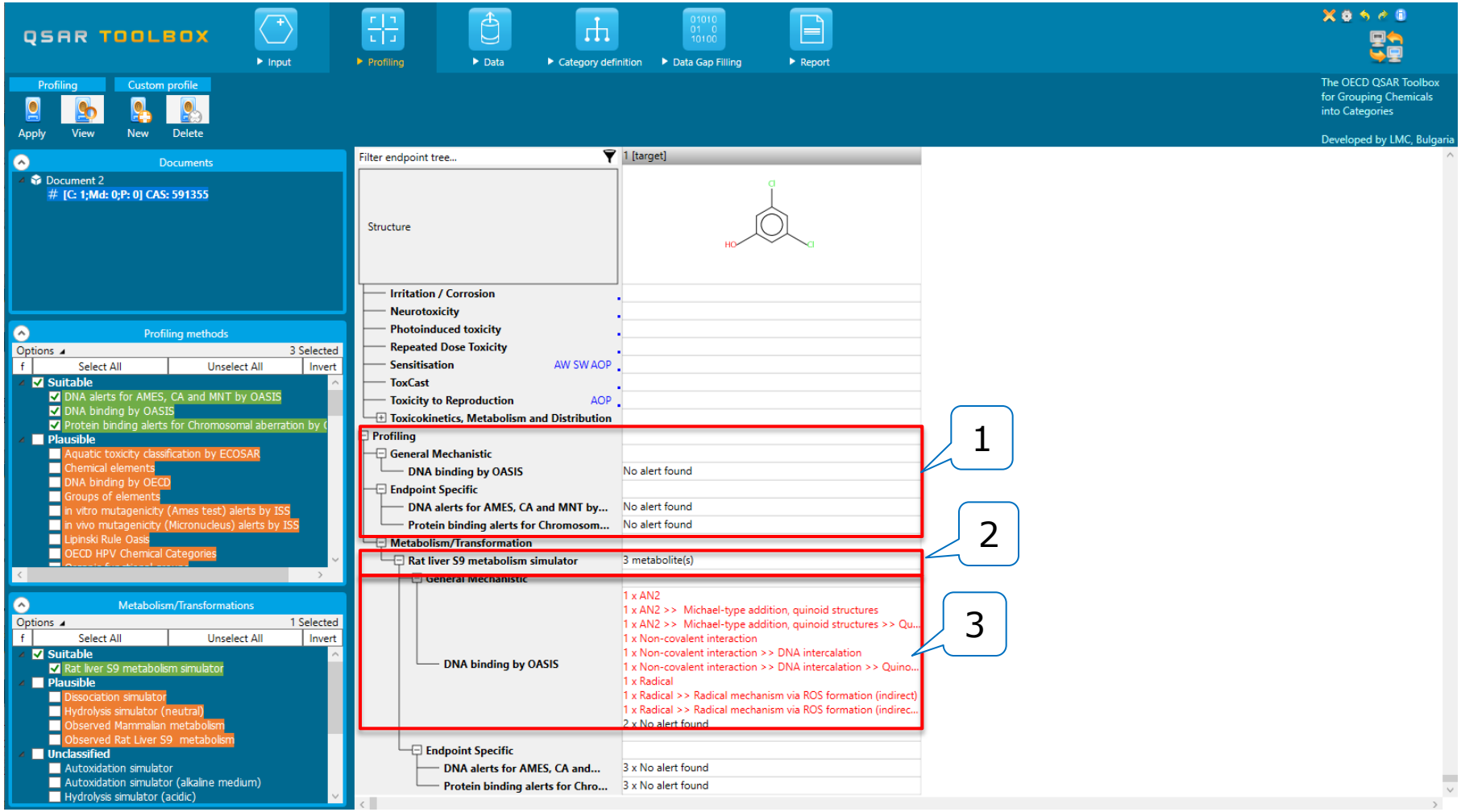
Profiling
Profiling results

1) No DNA and Protein binding alerts for chromosomal aberration are identified in the target structure as a parent;
2) 3 metabolites are produced as a result of Rat liver S9 metabolism simulator;
3) General mechanistic DNA binding alerts are identified in the metabolites produced by the Rat liver S9 metabolism simulator.

See on the next slide

Profiling

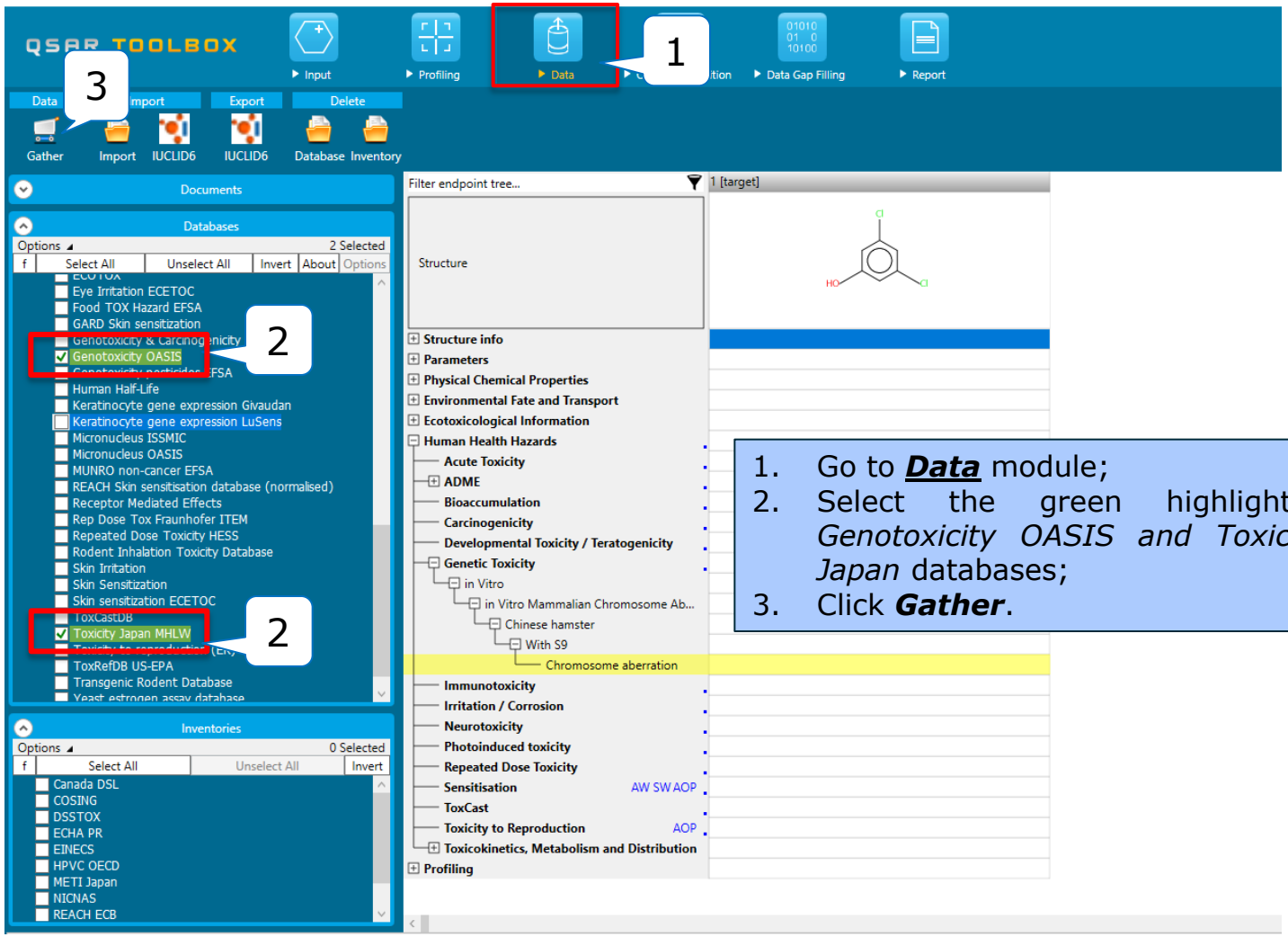
Profiling the target chemical



Data
Overview

- "Data" refers to the electronic process of retrieving the environmental fate, ecotoxicity and toxicity data that are stored in the Toolbox;
- Data gathering can be executed in a global fashion (i.e., collecting all data for all endpoints) or on a more narrowly defined basis (e.g., collecting data for a single or limited number of endpoints).

Data
Collecting experimental data

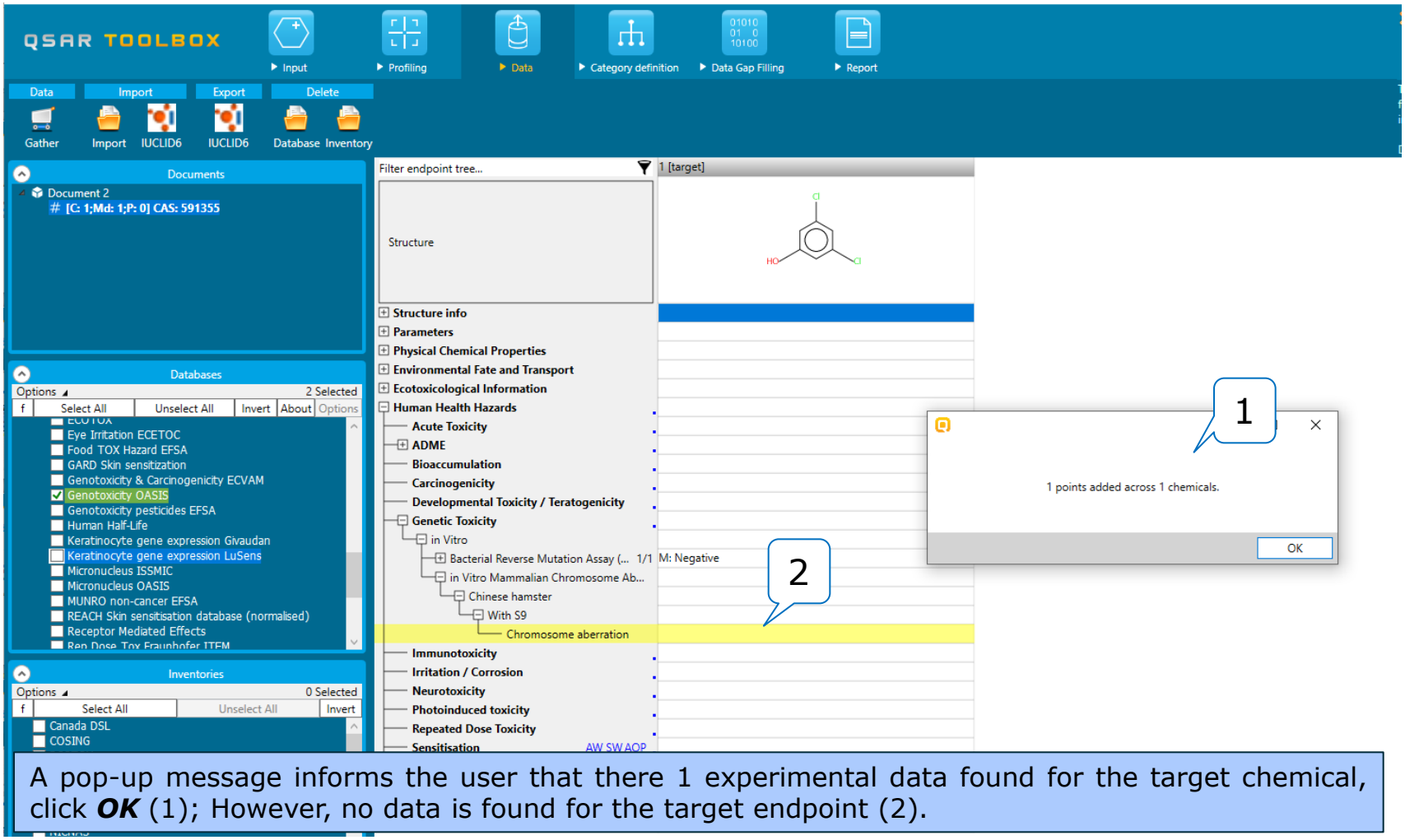


1. Go to ***Data*** module;
2. Select the green highlighted *Genotoxicity OASIS and Toxicity Japan* databases;
3. Click ***Gather***.

Data
Collecting experimental data

- Toxicity information on the target chemical is electronically collected from the selected dataset(s).
- It should be kept in mind that the search for data and analogues is performed only among the chemicals which are listed in the selected database(s), which in this example are *Genotoxicity OASIS and Toxicity Japan MHLW.*
- No experimental data related to the target endpoint are found (see next slide).

Data
Collecting experimental data



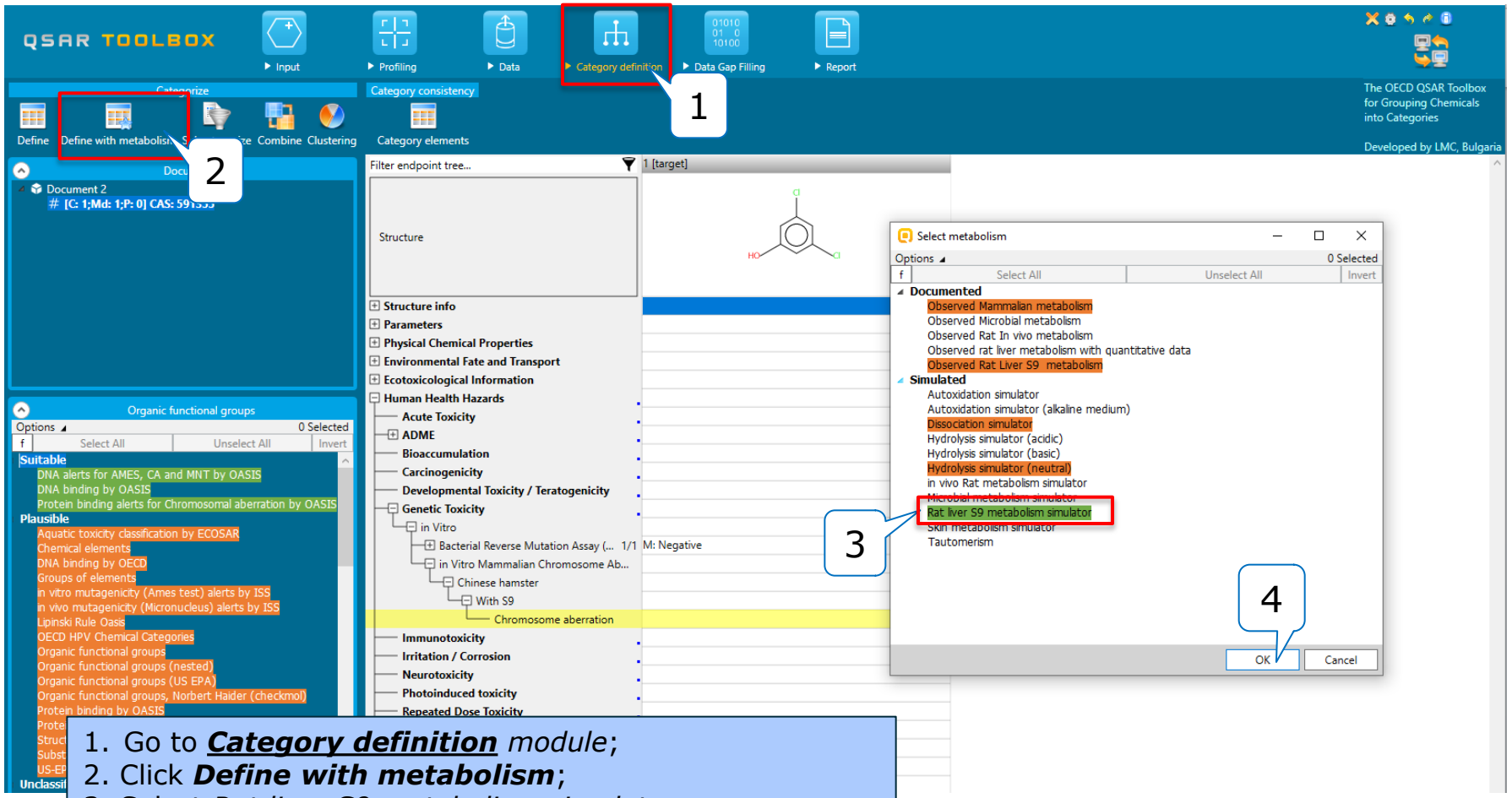
A pop-up message informs the user that there 1 experimental data found for the target chemical, click ***OK*** (1); However, no data is found for the target endpoint (2).

Category Definition
Overview

- This module provides the user with several means of grouping chemicals into a toxicologically meaningful category that includes the target molecule.
- This is the critical step in the workflow.
- Several options are available in the Toolbox to assist the user in refining the category definition.
- In this case no DNA alert is identified in the target structure, but in its metabolites produced after *in vitro* rat liver metabolic activation. Based on that the analogues will be searched accounting for *in vitro* rat liver metabolism. In this way the target chemical and the identified analogues will have similar metabolic pattern (see the next slides).

Category Definition

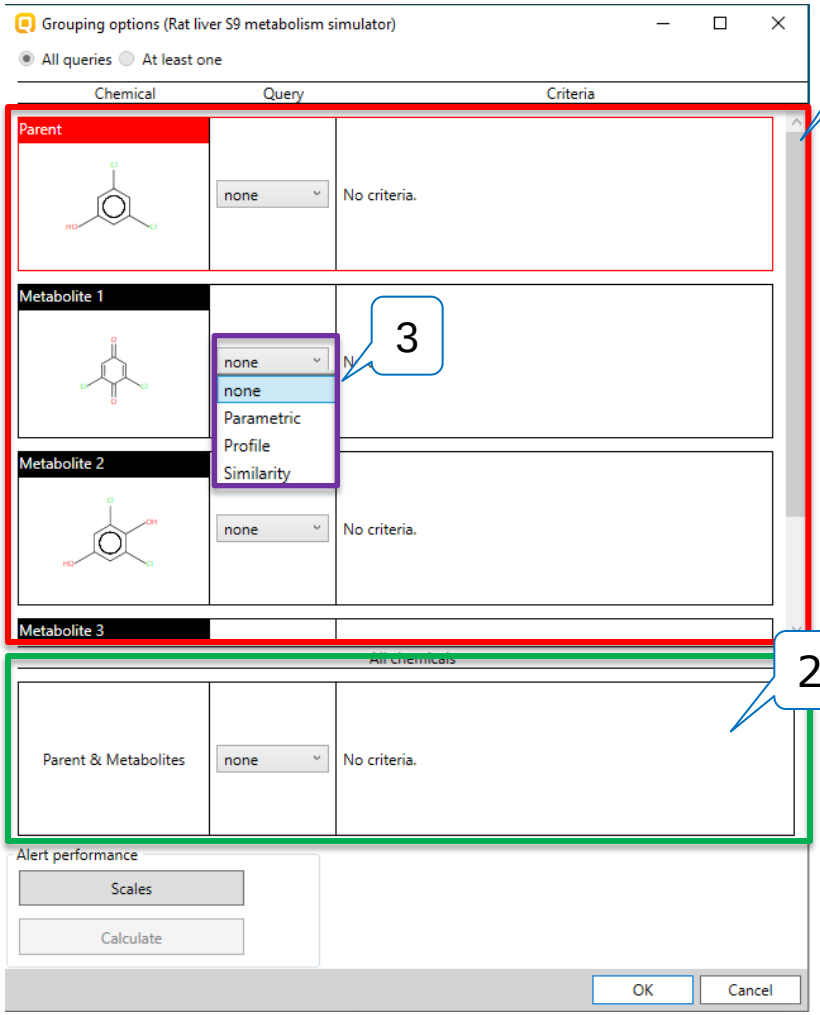
Searching for analogues accounting for *rat in vitro* metabolism



1. Go to ***Category definition*** *module*;
2. Click ***Define with metabolism***;
3. Select *Rat liver S9 metabolism simulator*;
4. Click ***OK.***

Category Definition

Searching for analogues accounting for *rat in vitro* metabolism



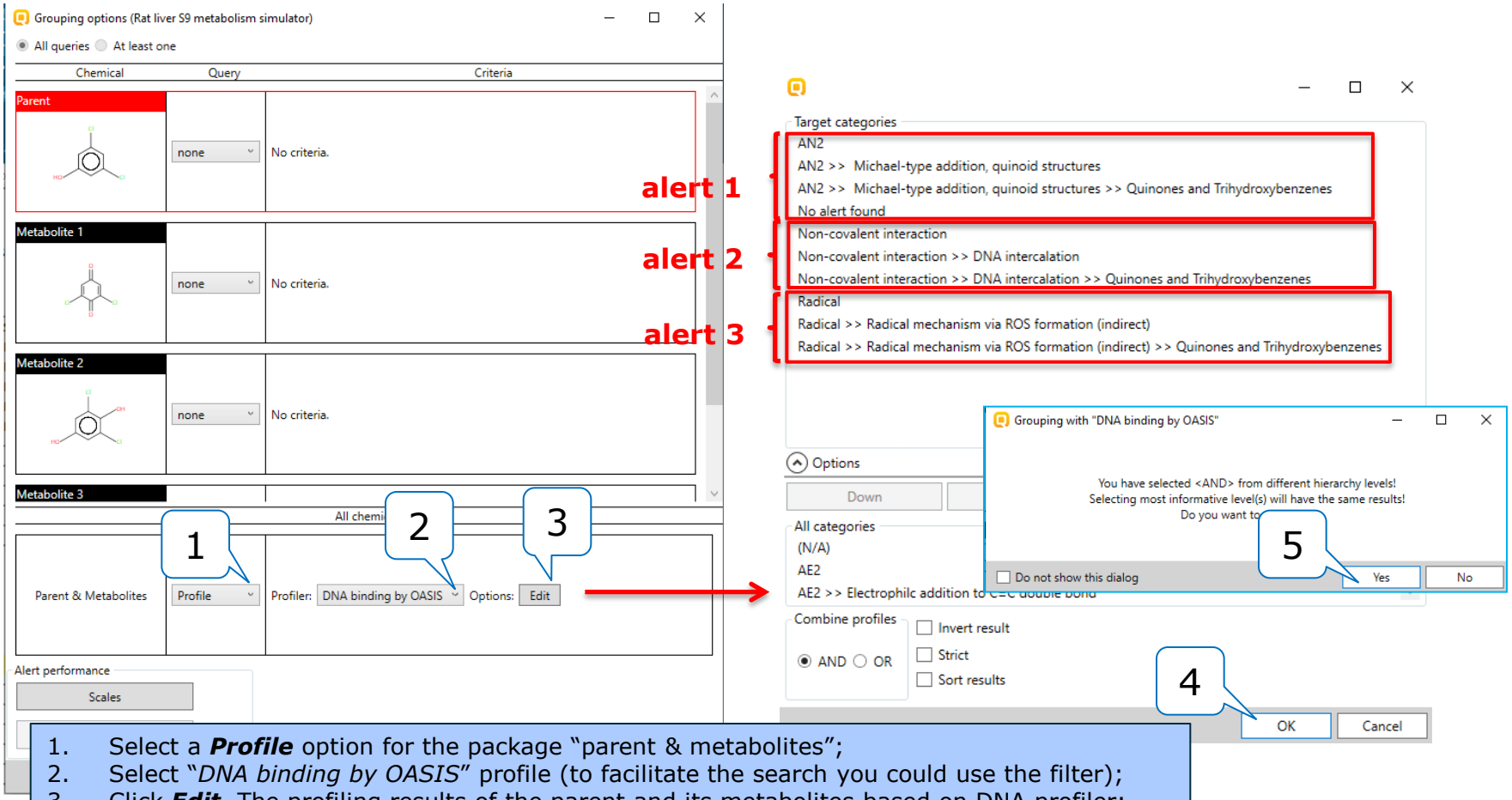
Grouping options dialogue appears. It shows all the generated metabolites of the target chemical (use the scroll bar to see them). It has two subsections:

- (1) shows the parent and each of the generated metabolites. This allows defining different criteria for each structure when looking for analogues.
- (2) treats the parent and its metabolites as a whole. i.e. the criteria is provided for the whole package (parent & metabolites) but not for separate metabolites.

A drop down menu (3) is available in the column "Query" for each of the structures which allow setting the type of criteria for looking for analogues.

Category Definition

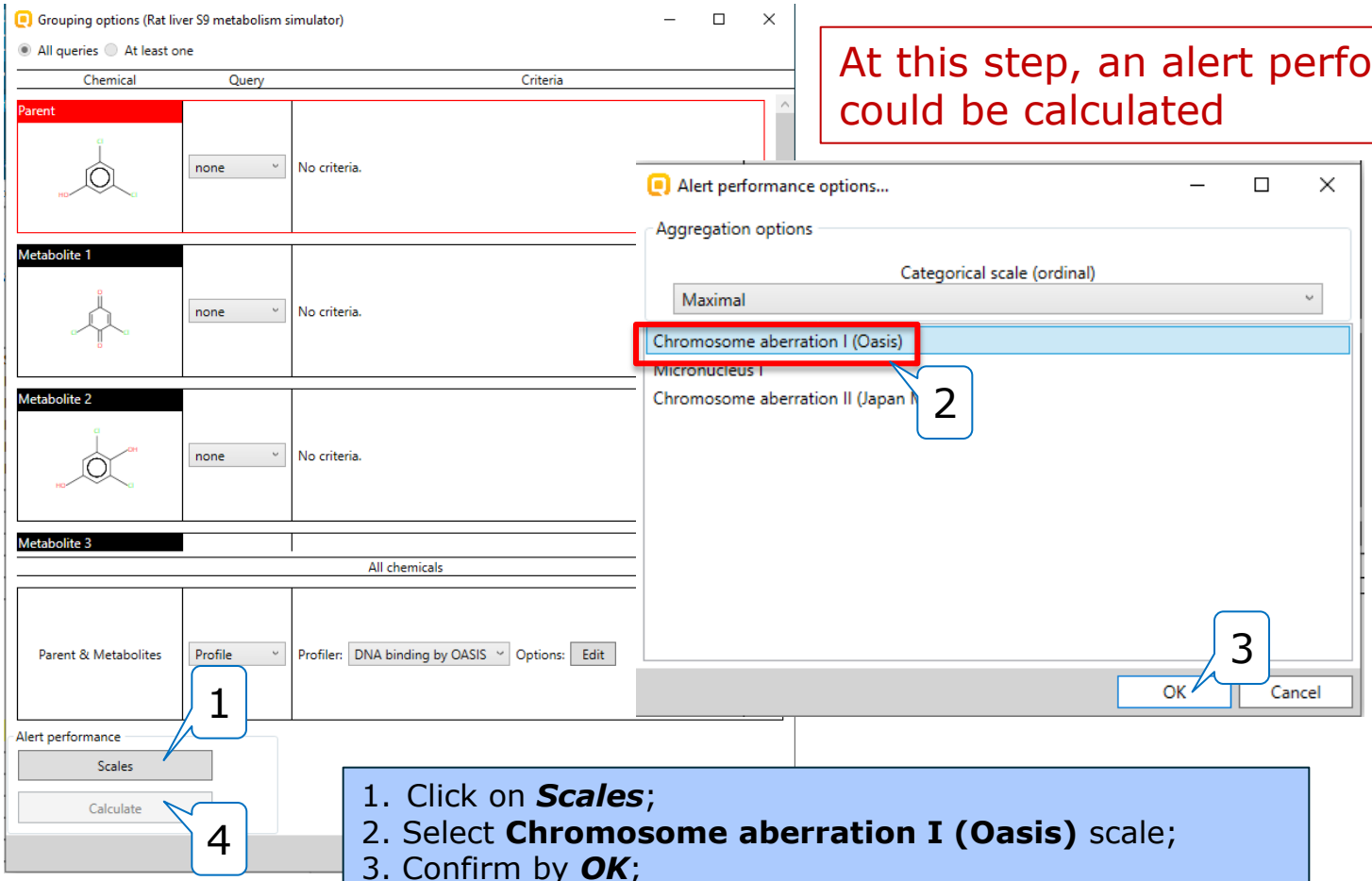
Searching for analogues accounting for *rat in vitro* metabolism



1. Select a ***Profile*** option for the package "parent & metabolites";
2. Select "*DNA binding by OASIS*" profile (to facilitate the search you could use the filter);
3. Click ***Edit***. The profiling results of the parent and its metabolites based on DNA profiler;
4. Click ***OK*** to confirm the defined search criteria.
5. Click ***OK*** on the appeared message.

Category Definition

Searching for analogues accounting for *rat in vitro* metabolism

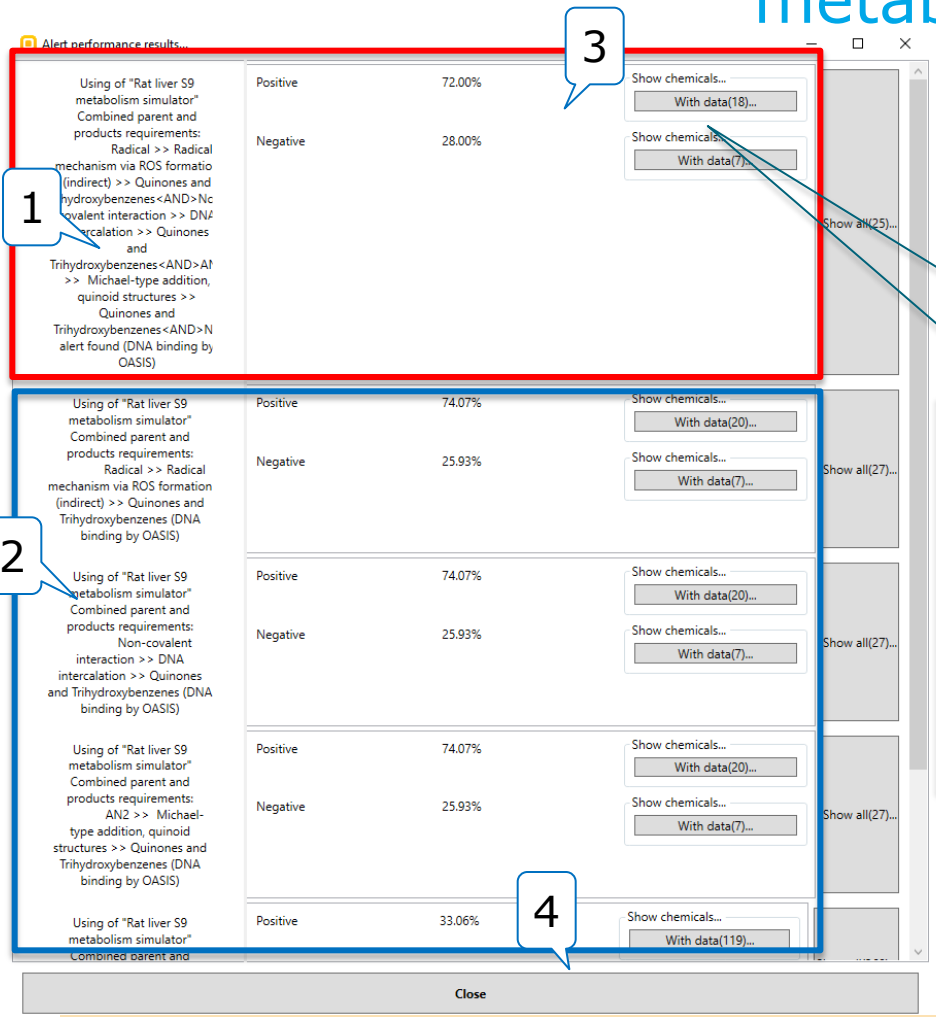


At this step, an alert performance could be calculated

1. Click on ***Scales***;
2. Select **Chromosome aberration I (Oasis)** scale;
3. Confirm by ***OK***;
4. Click ***Calculate***.

Category Definition

Searching for analogues accounting for *rat in vitro* metabolism

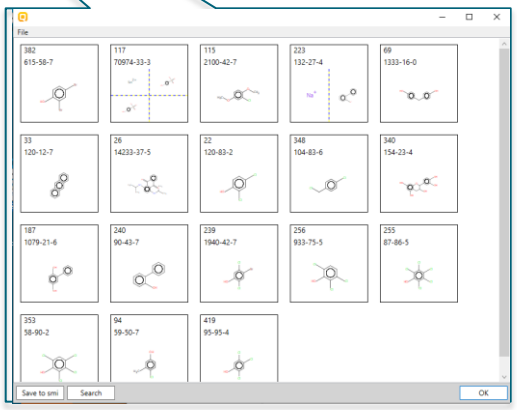


Once the calculation of AP is finished, a new window appears providing the following information:

1) AP statistic accounting for all set criteria and all identified alerts in case of selected *profile* query.

2) AP statistic for each of the searching criteria (i.e. for each of the alerts)

3) Percentages of different data (positive/negative) and number of chemicals used for the statistic. The user is also able to see the corresponding chemicals (the parent chemicals are shown, only).



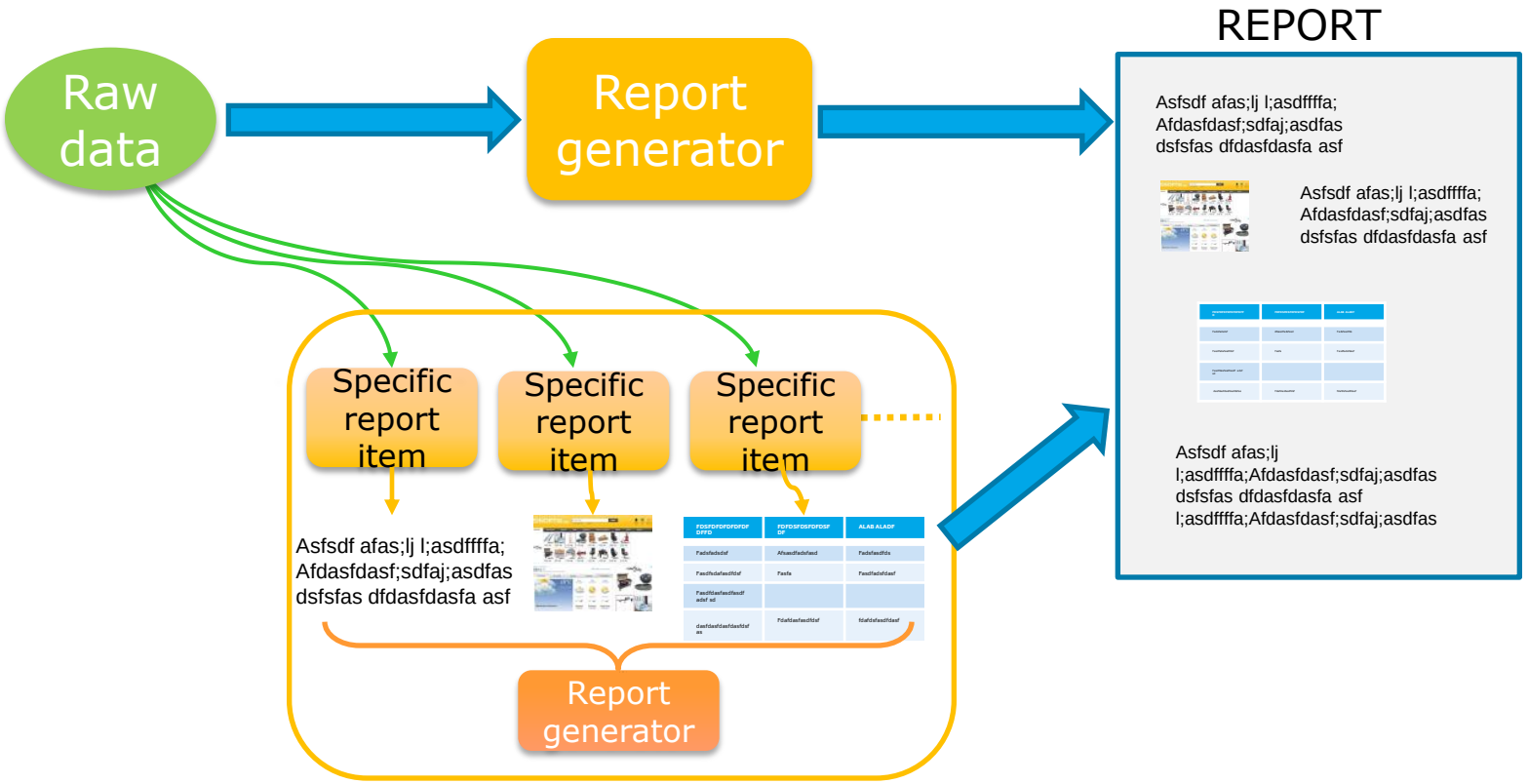
The performance shows that the *Quinones and Trihydroxybenzenes* alert has very high predictability with respect the defined endpoint and selected databases.

After analyzing the provided information close the window (4).

Calculation of alert performance create a specific report item stored in the so-called *Report basket*.

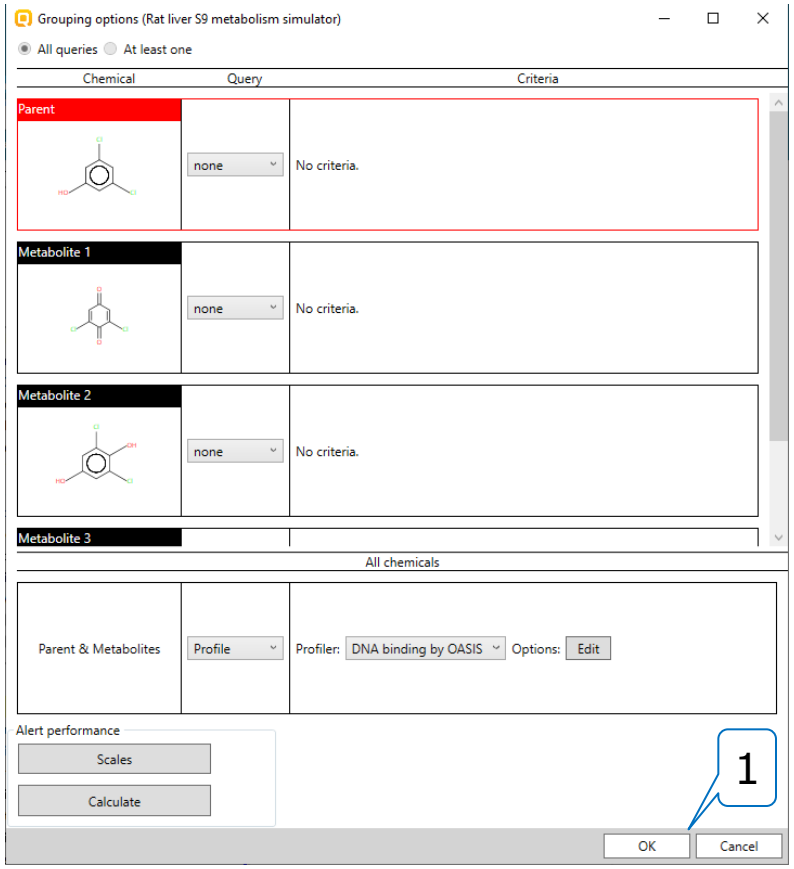
Report basket - overview

- The specific report items are collected during the workflow or from external modeling sources.
- All items are stored in the "*Report basket*" and can be used in the report to support or justify the consistency of a category.



Category Definition

Searching for analogues accounting for *rat in vitro* metabolism

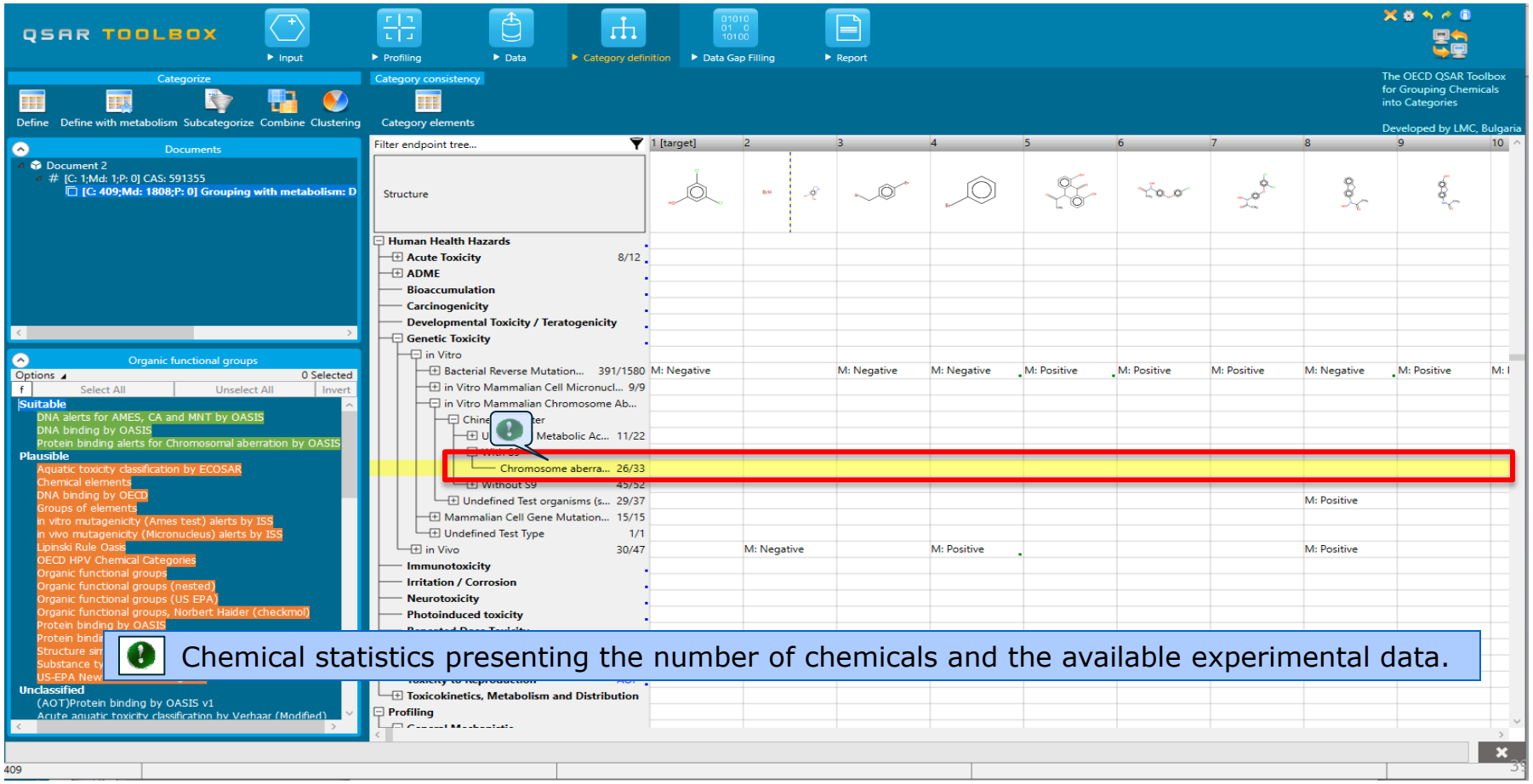


After closing the *Alert performance* window, click ***OK*** (1) in Grouping options window to execute the search. The Toolbox system will search within the selected database for chemicals having the same metabolic pattern with respect to *DNA binding by OASIS profiler* as the target chemical.

Category Definition

Searching for analogues accounting for *rat in vitro* metabolism

26 chemicals (out of 408 chemicals) with 33 experimental results are identified fulfilling the searched criteria
The result might be different, in case other selection of databases and grouping methods are used.



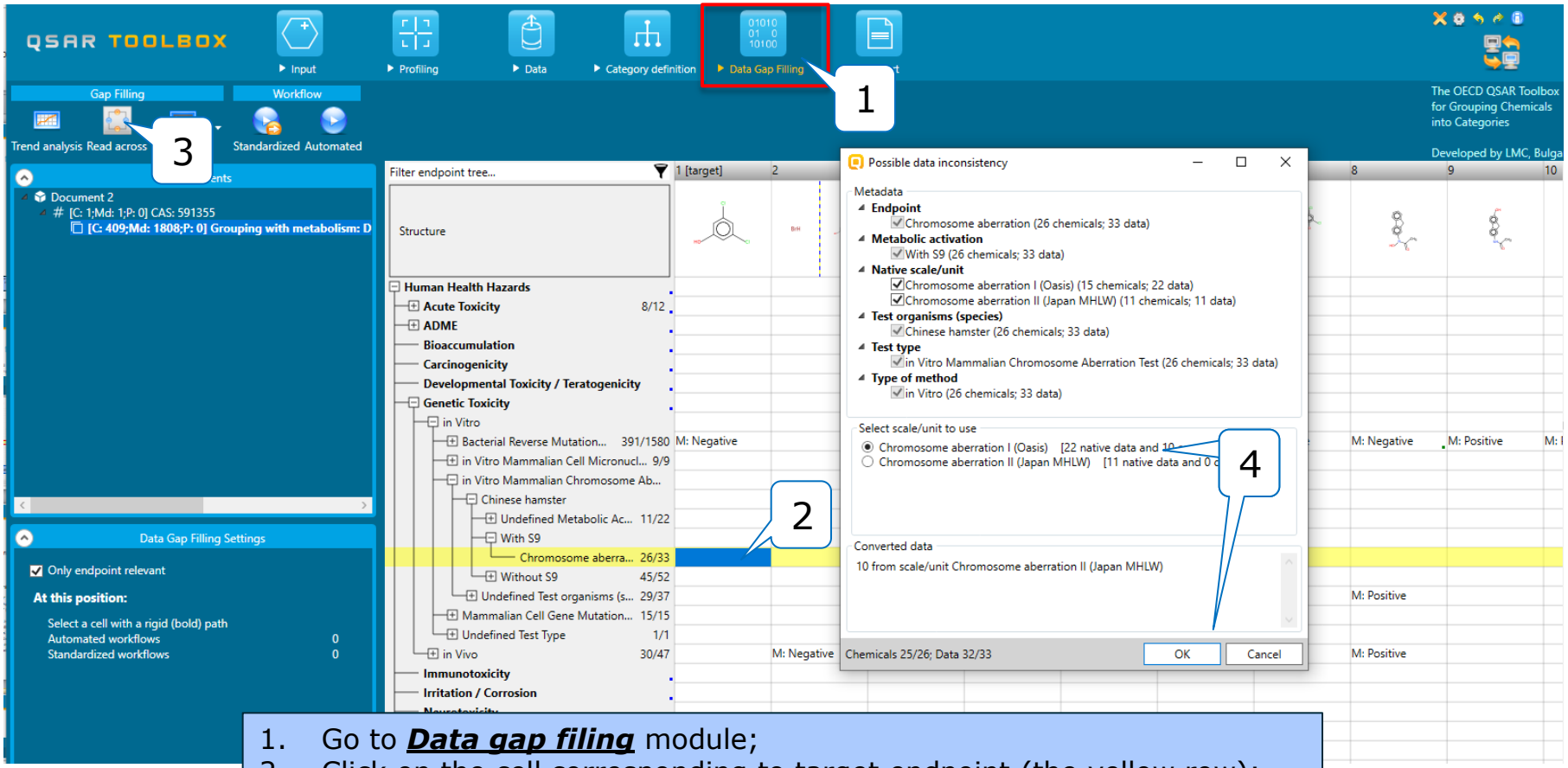
Data Gap Filling
Overview

- "Data Gap Filling" module give access to five different data gap filling tools:
  - Read-across
  - Trend analysis
  - (Q)SAR models
  - Standardized workflow
  - Automated workflow
- Depending on the situation, the most relevant data gap mechanism should be chosen, taking into account the following considerations:
  - Read-across is the appropriate data-gap filling method for "qualitative" endpoints like skin sensitisation or mutagenicity for which a limited number of results are possible (e.g. positive, negative, equivocal). Furthermore read-across is recommended for "quantitative endpoints" (e.g., 96h-LC50 for fish) if only a low number of analogues with experimental results are identified.
  - Trend analysis is the appropriate data-gap filling method for "quantitative endpoints" (e.g., 96h-LC50 for fish) if a high number of analogues with experimental results are identified.
  - "(Q)SAR models" can be used to fill a data gap if no adequate analogues are found for a target chemical.

  Additionally, two workflows - Standardized and Automated have been developed in order to facilitate the users work. Once started, they follow the implemented logic and finish with prediction. The general differences between the two type of workflows are represented on the next slide.

In this example we will use read-across approach.

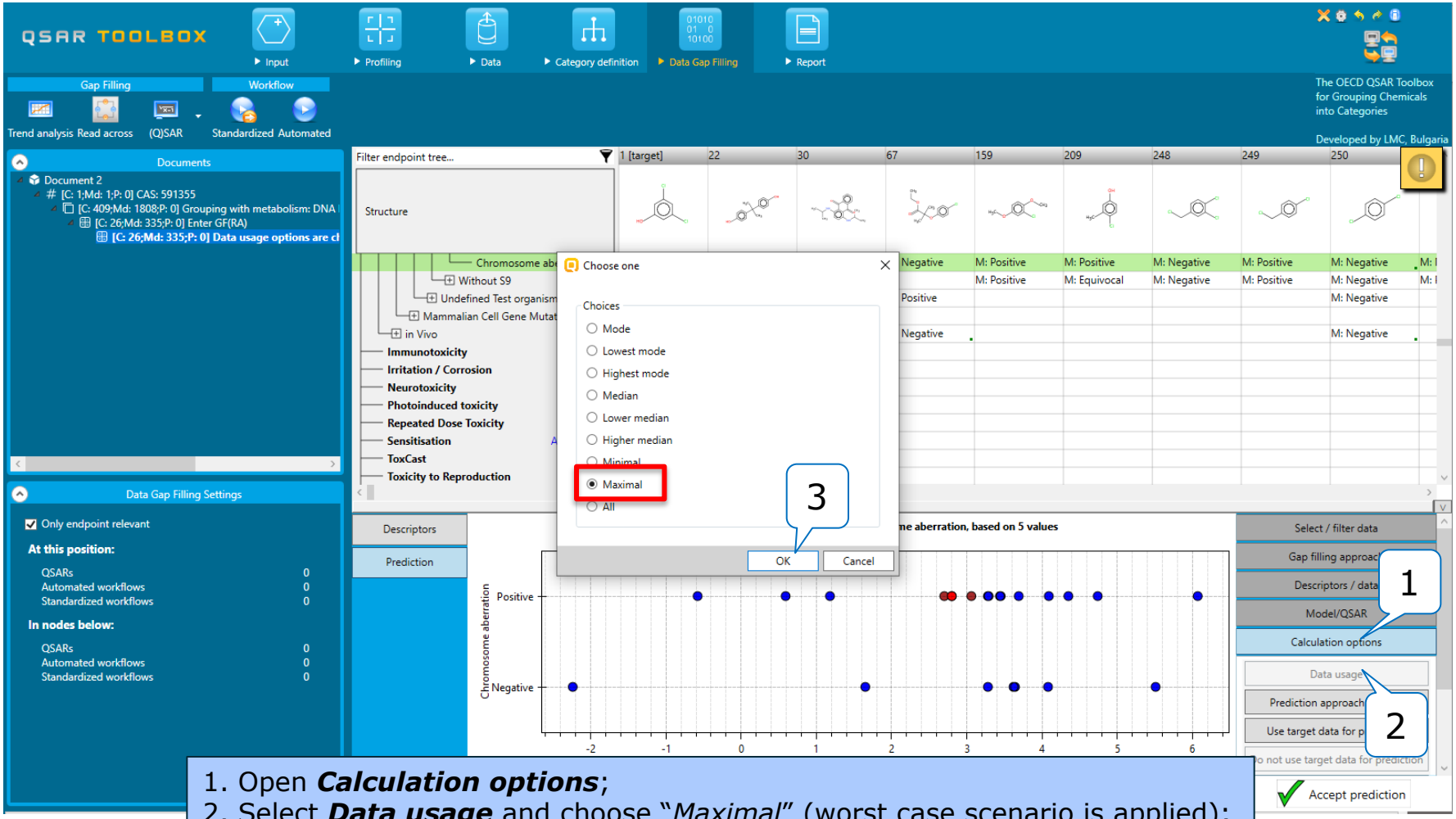
Data Gap Filling
Apply Read-across



1. Go to ***Data gap filing*** module;
2. Click on the cell corresponding to target endpoint (the yellow row);
3. Click ***Read across***.
4. Select scale **Chromosomal aberration I (OASIS)** and click **OK**

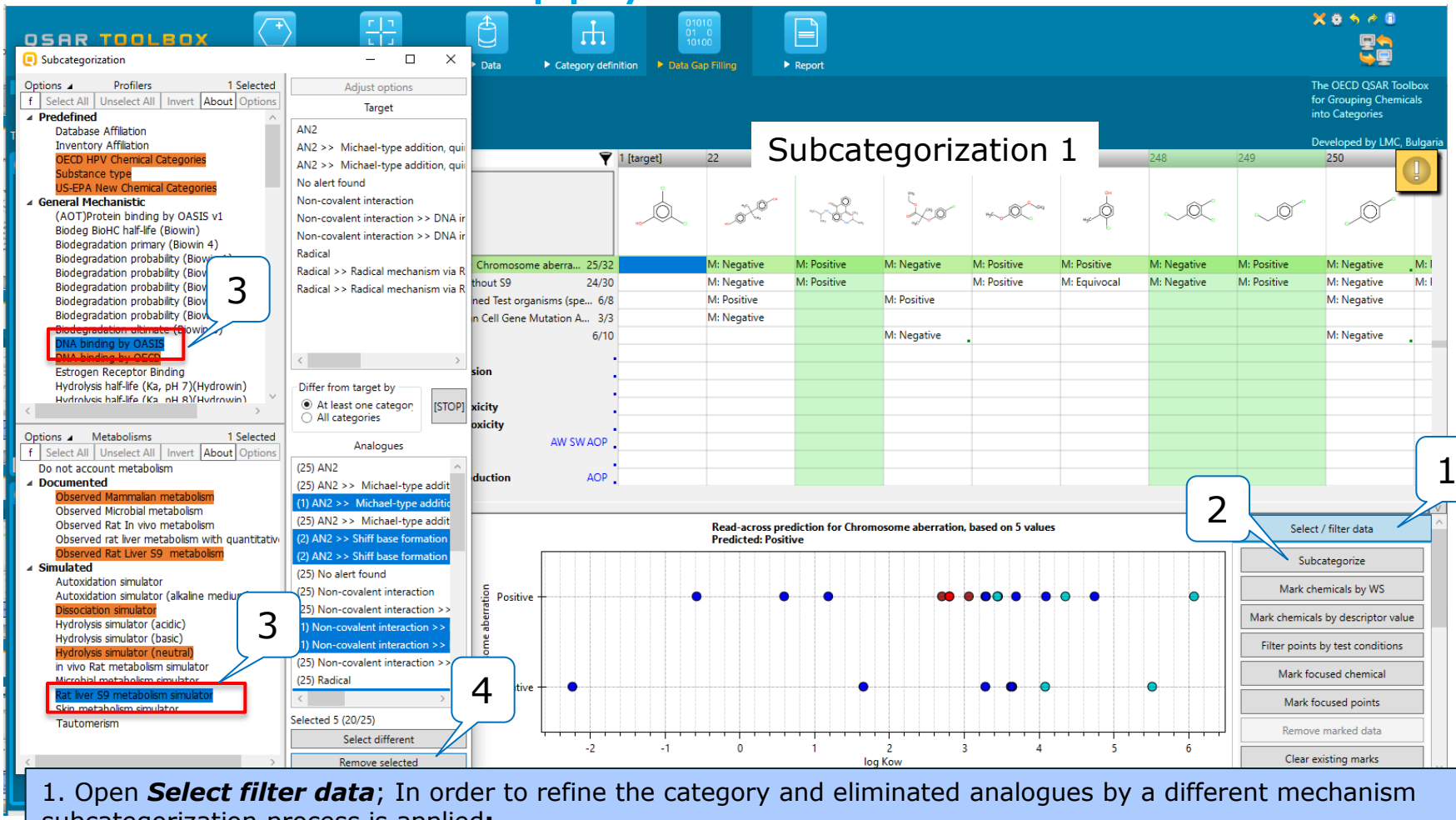
Data Gap Filling

Apply Read-across



1. Open ***Calculation options***;
2. Select ***Data usage*** and choose "*Maximal*" (worst case scenario is applied);
3. Select ***OK***.

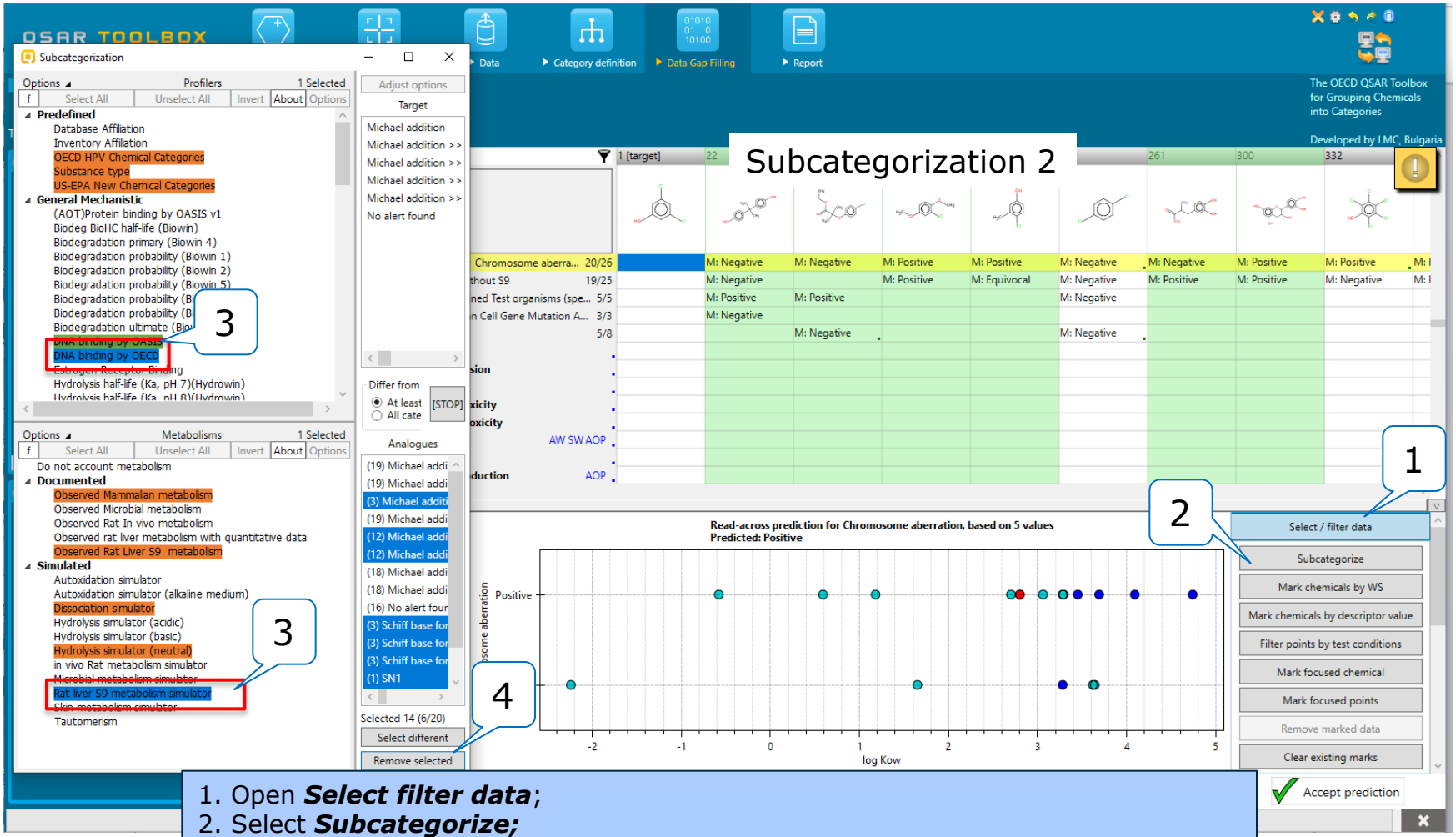
Data Gap Filling
Apply Read-across



1. Open ***Select filter data***; In order to refine the category and eliminated analogues by a different mechanism subcategorization process is applied**:**
2. Select ***Subcategorize;***
3. Apply *DNA binding by OASIS* combined with *Rat liver S9 metabolism simulator*;
4. **Remove** analogues reacting by different mechanisms than the target chemical.

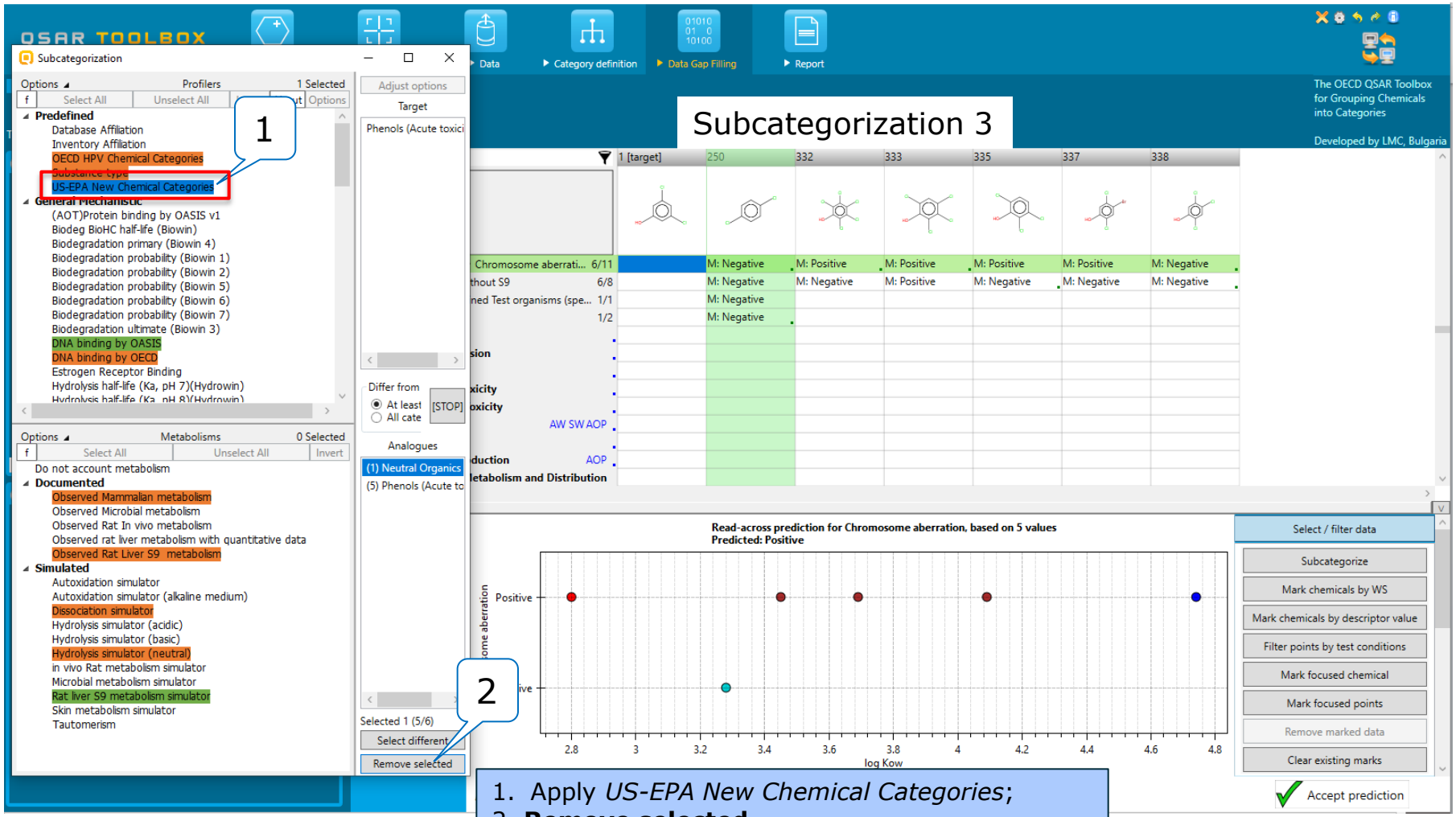
Data Gap Filling

Apply Read-across



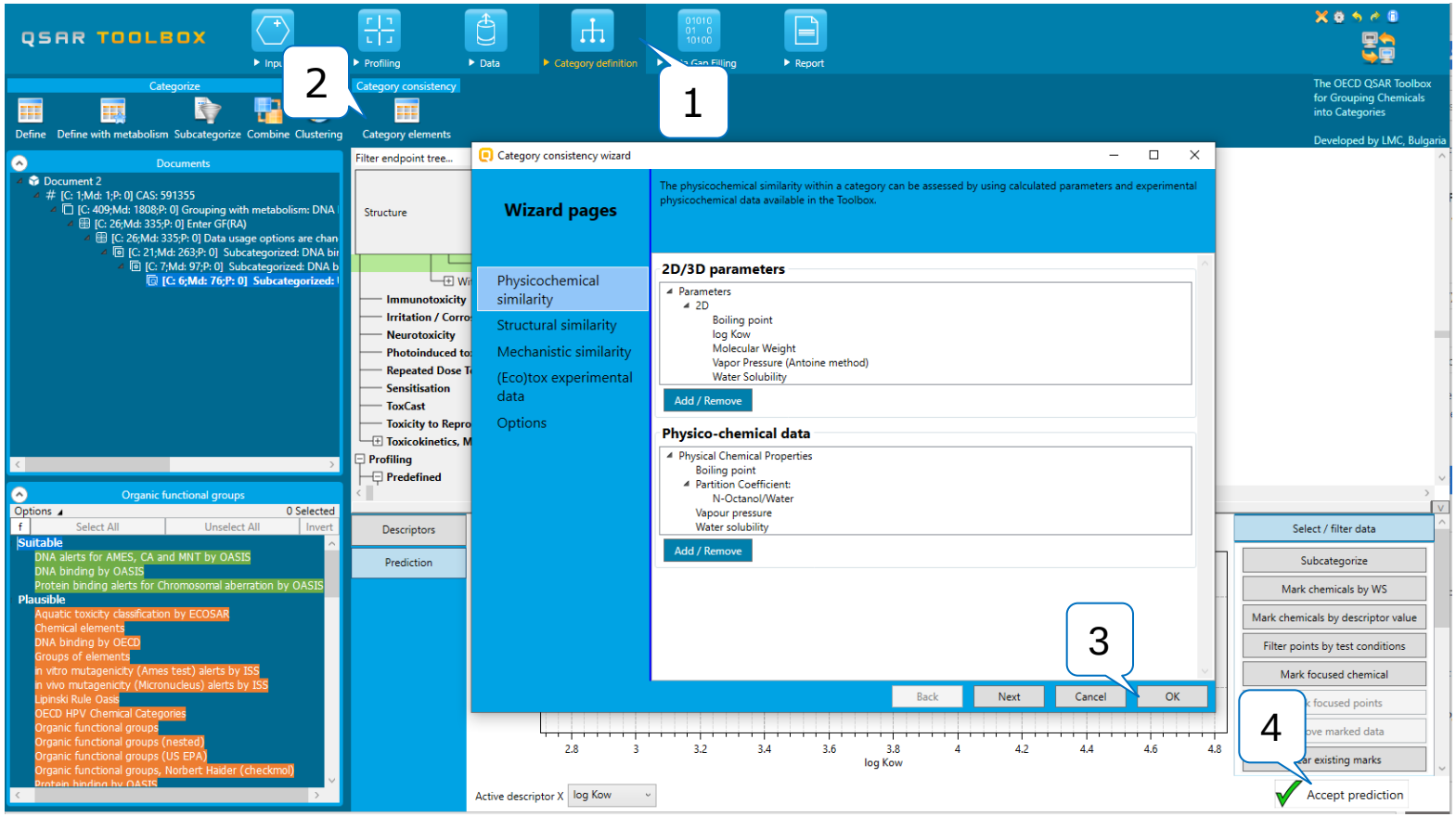
1. Open ***Select filter data***;
2. Select ***Subcategorize;***
3. Apply *DNA binding by OECD* combined with *Rat liver S9 metabolism simulator*;
4. **Remove** analogues reacting by different mechanisms than the target chemical

Data Gap Filling
Apply Read-across



Data Gap Filling

Apply Category consistency elements



After subcategorization process go back go the ***Category definition*** module (1) and apply ***Category elements**** (2). No different selection than the default is needed – click ***OK*** (3). Once the category elements are applied ***Accept prediction*** (4).

*For more information on category elements see *Tutorial TB 4.4. Category consistency*

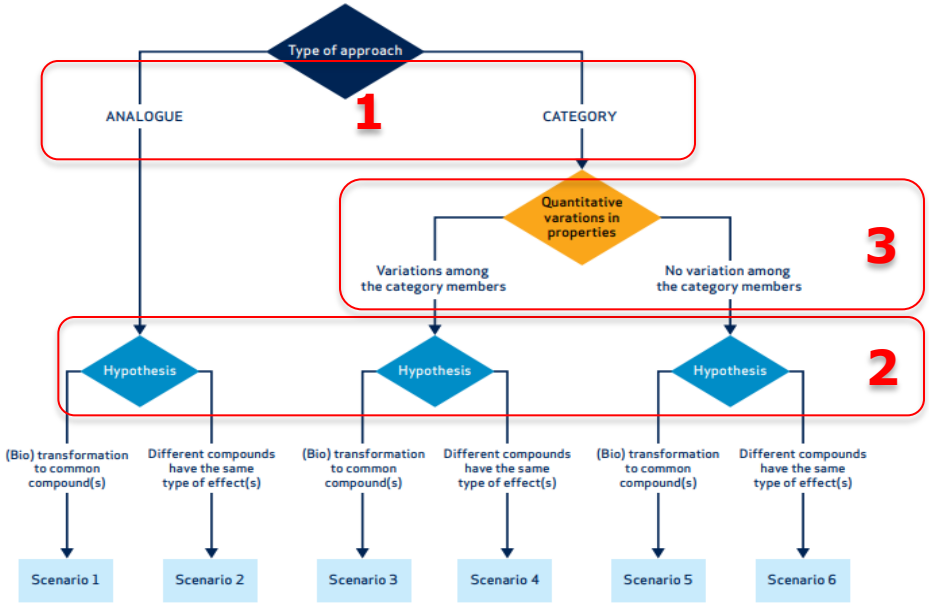
Report
Overview

- The report module allows generating a report for predictions performed within the Toolbox.
- The report module contains a predefined report template which users can customize.
- Additionally specific RAAF scenario could be chosen. Selection of one of the scenarios will append automatically the related assessment elements (AE) related to the corresponding report sections.
- For fulfilling of the AEs' sections an information stored in the so called "report basket" will be of help (see next slides)

Report
Considerations for selection of RAAF scenario

To select the applicable RAAF scenario for assessment, the following aspect should be taken into account:

1) the type of approach applied - analogue approach or category approach;
2) the read-across hypothesis – (bio)transformation to common compound or different compounds have the same type of effect(s)
3) For category approach - whether quantitative variations in the properties are observed among the category members must be considered.



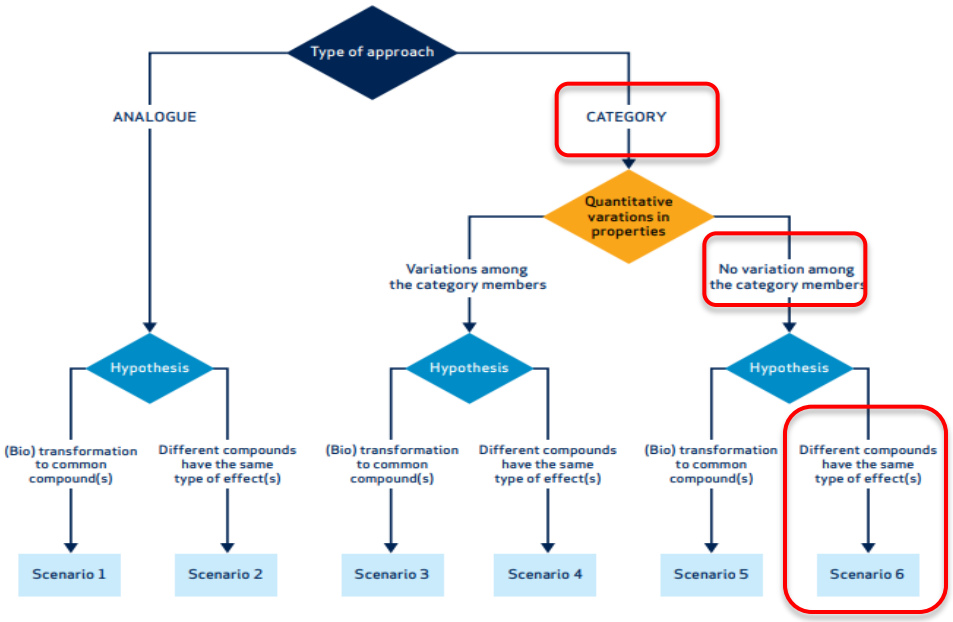
*Read-Across Assessment Framework (RAAF) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

Report
Selection of RAAF scenario

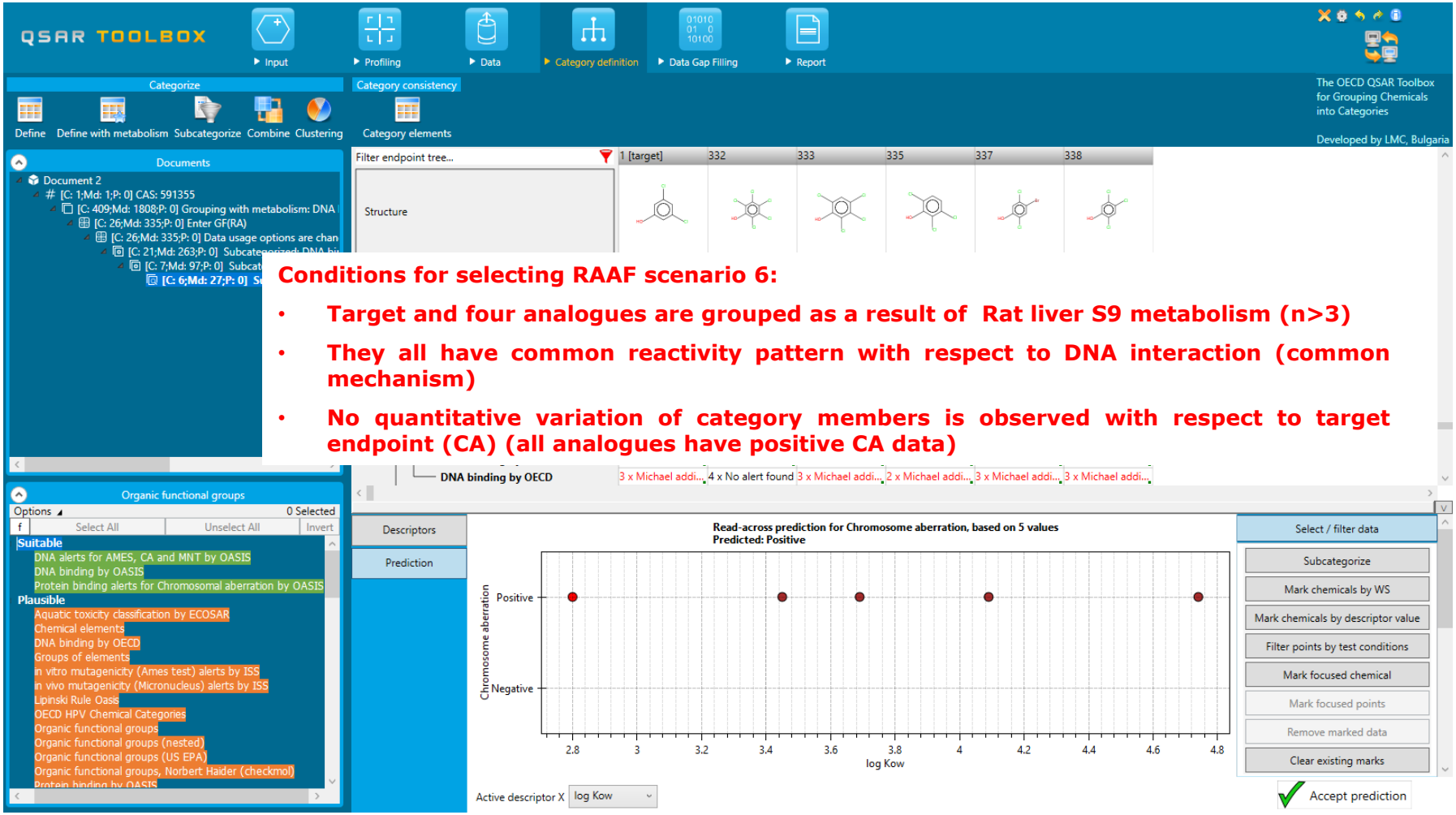
For the current example:

1) the type of approach applied - category approach is used (threshold of >3 analogues);
2) the read-across hypothesis – different compounds with common underlying mechanism;
3) For category approach – no quantitative variations is observed among the category members

Based on that Scenario 6 was selected for the current example.



Report
Selection of RAAF scenario

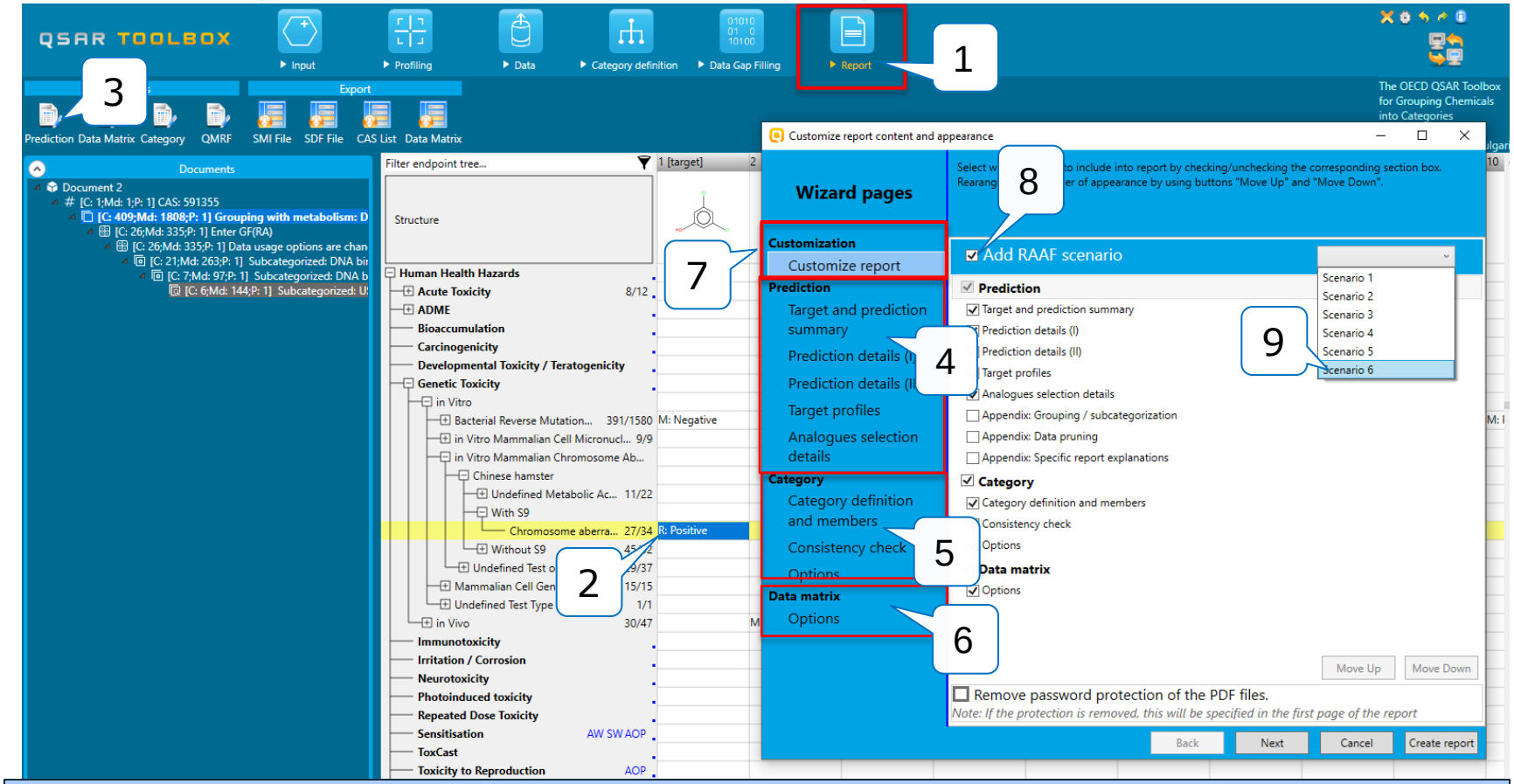


Conditions for selecting RAAF scenario 6:

- **Target and four analogues are grouped as a result of Rat liver S9 metabolism (n>3)**
- **They all have common reactivity pattern with respect to DNA interaction (common mechanism)**
- **No quantitative variation of category members is observed with respect to target endpoint (CA) (all analogues have positive CA data)**

Report

Report generation according to RAAF Scenario 6



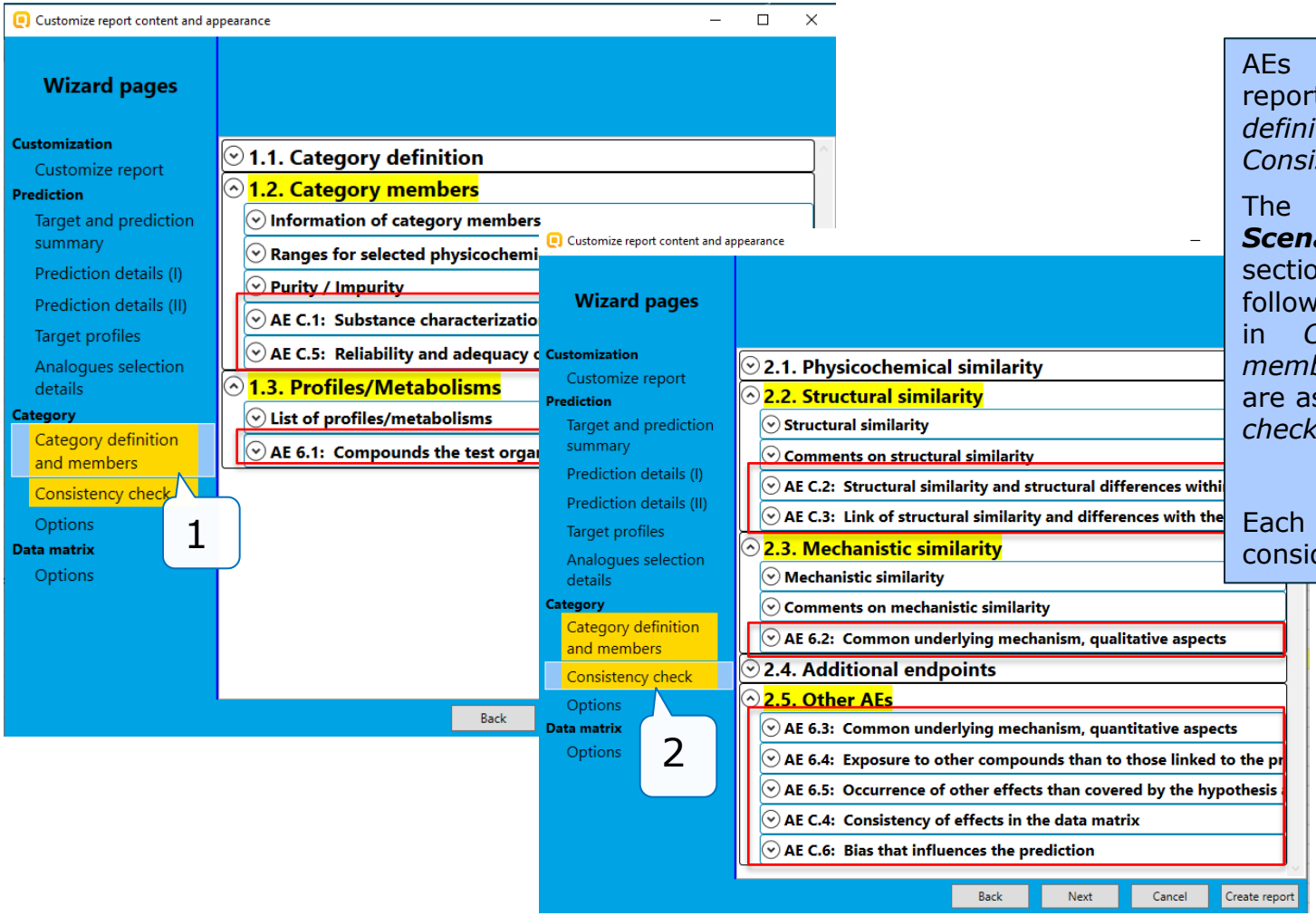
1. Go to ***Report*** module;
2. Select a cell with prediction – ***"R: Positive"***;
3. Click the ***Prediction*** button.

The ***Report wizard*** appears. It consists of different sections related to the types of the report - ***Prediction*** (4), ***Category*** (5) and ***Data matrix*** (6).The content of each of these three sections could be customized in the ***Customization*** section *(7);*
Check the box at the top to add **RAAF scenario** (8). Select **Scenario 6** from the drop-down menu (9).

Report

Report generation according to RAAF Scenario 6

AEs related to each scenario appeared automatically to the corresponding report section



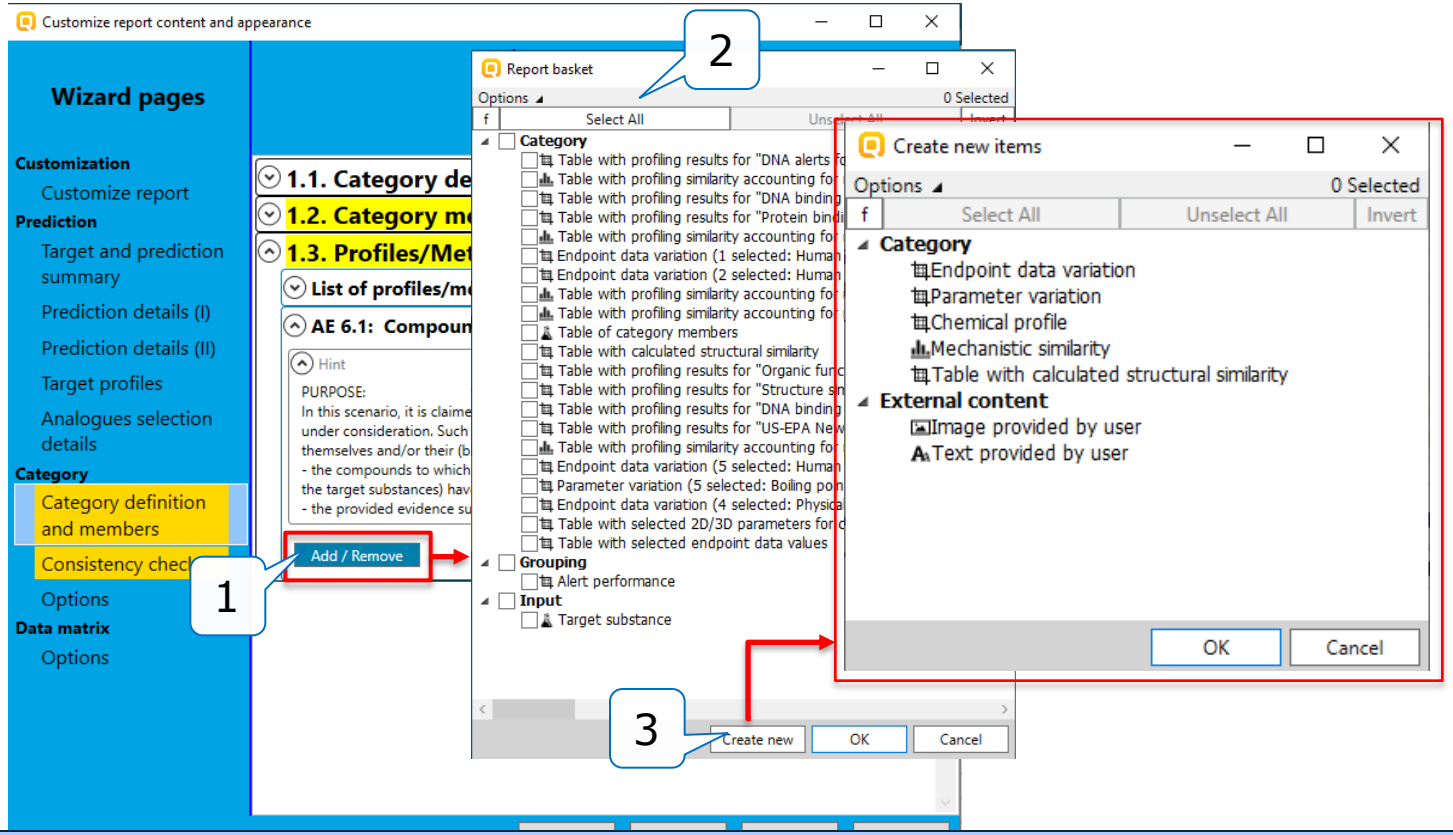
AEs appear in the following report sections: *Category definition and members* (1) and *Consistency check* (2).

The assessment elements of ***Scenario 6*** are distributed in sections mentioned above as follows: three AEs are included in *Category definition and members* (1), and eight AEs are associated with *Consistency check* (2).

Each of the AEs will be considered in the next slides.

Report

Report generation according to RAAF Scenario 6



Information can be included by clicking the **Add/Remove** button (1) located below the corresponding AE. The *Add/Remove* button invokes the so-called "***Report basket***" (2). The latter contain different items triggered by the actions of the user during the workflow (e.g. Alert performance calculation, applying of category elements, etc.).
Additionally, new items (including items with external content) can be created (3).

Items with external content (text and picture) will be added for **AE 6.1: Compounds the test organism is exposed to (see next slides).**

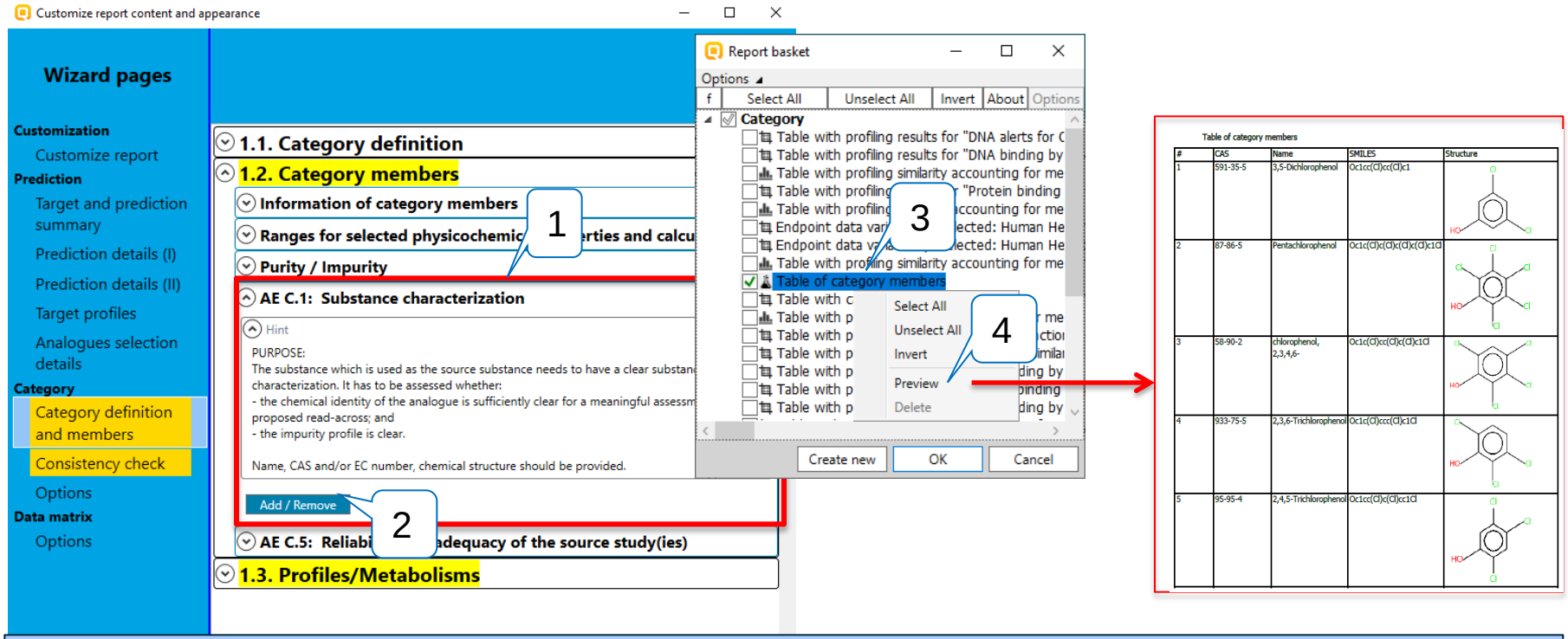
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Category definition and members*

AE C.1: *Substance characterization*



One assessment element (AE C.1) (1) related to the characterization of the category members is included in the ***Category definition and members*** section. In order to add substance characterization select ***Add/Remove*** button (2) and check *Table of category members* item (3), which is generated during the workflow. If impurities/additives of the used analogues are available, they will be also included. The current analogues have no additives/impurities. Example on how the AE C.1. will look in the generated report is shown when click on ***Preview*** button (4).

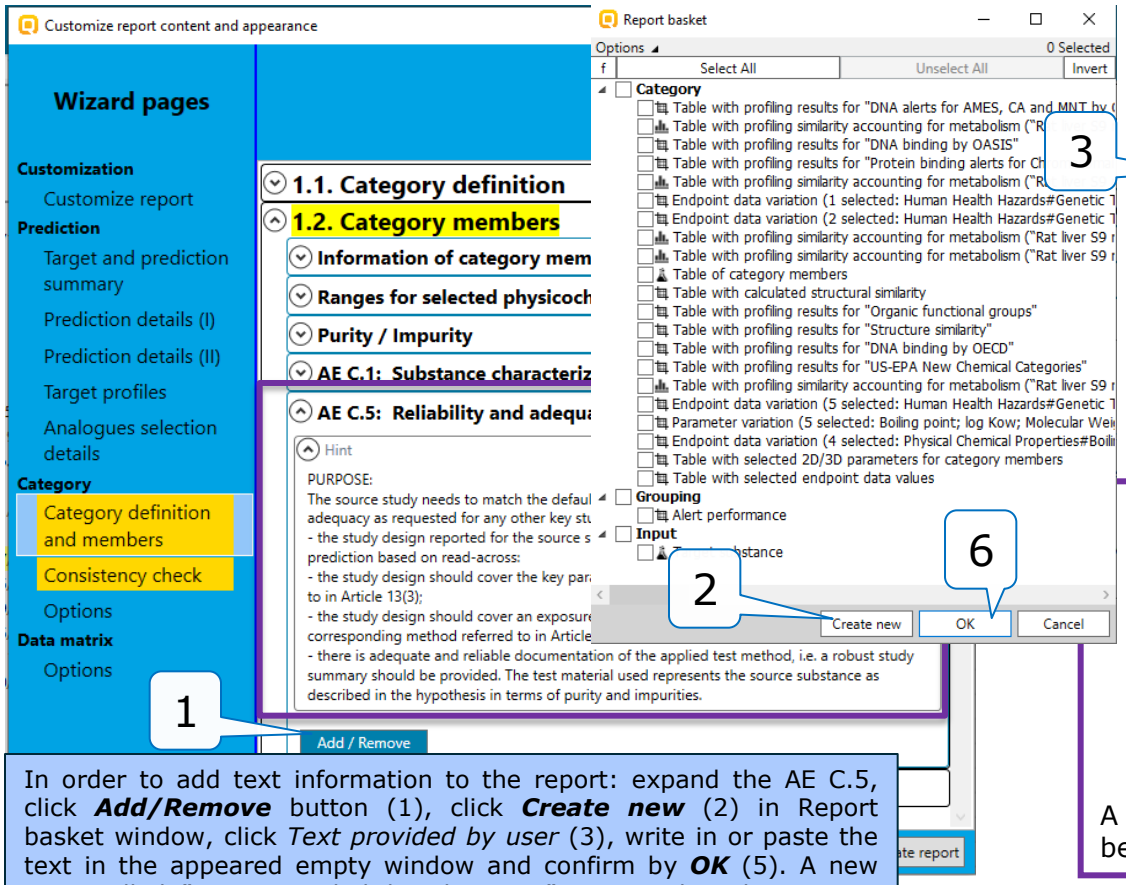
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Category definition and members*

AE C.5: *Reliability and adequacy of the source study(ies)*



In order to add text information to the report: expand the AE C.5, click ***Add/Remove*** button (1), click ***Create new*** (2) in Report basket window, click *Text provided by user* (3), write in or paste the text in the appeared empty window and confirm by ***OK*** (5). A new item called "Test provided by the user" appeared under section *External content* of the *Report basket* . Finally click ***OK*** *(6)*.

The possible example text is:

The target substance have been tested according to *in Vitro Mammalian Chromosome Aberration Test*

All the experimental data for four source substances has been tested based on *in Vitro Mammalian Chromosome Aberration Test and* are used for the prediction of Chromosomal aberration of the target substance A

A snapshot from *Filter points by test conditions* could be provided (see next slide).

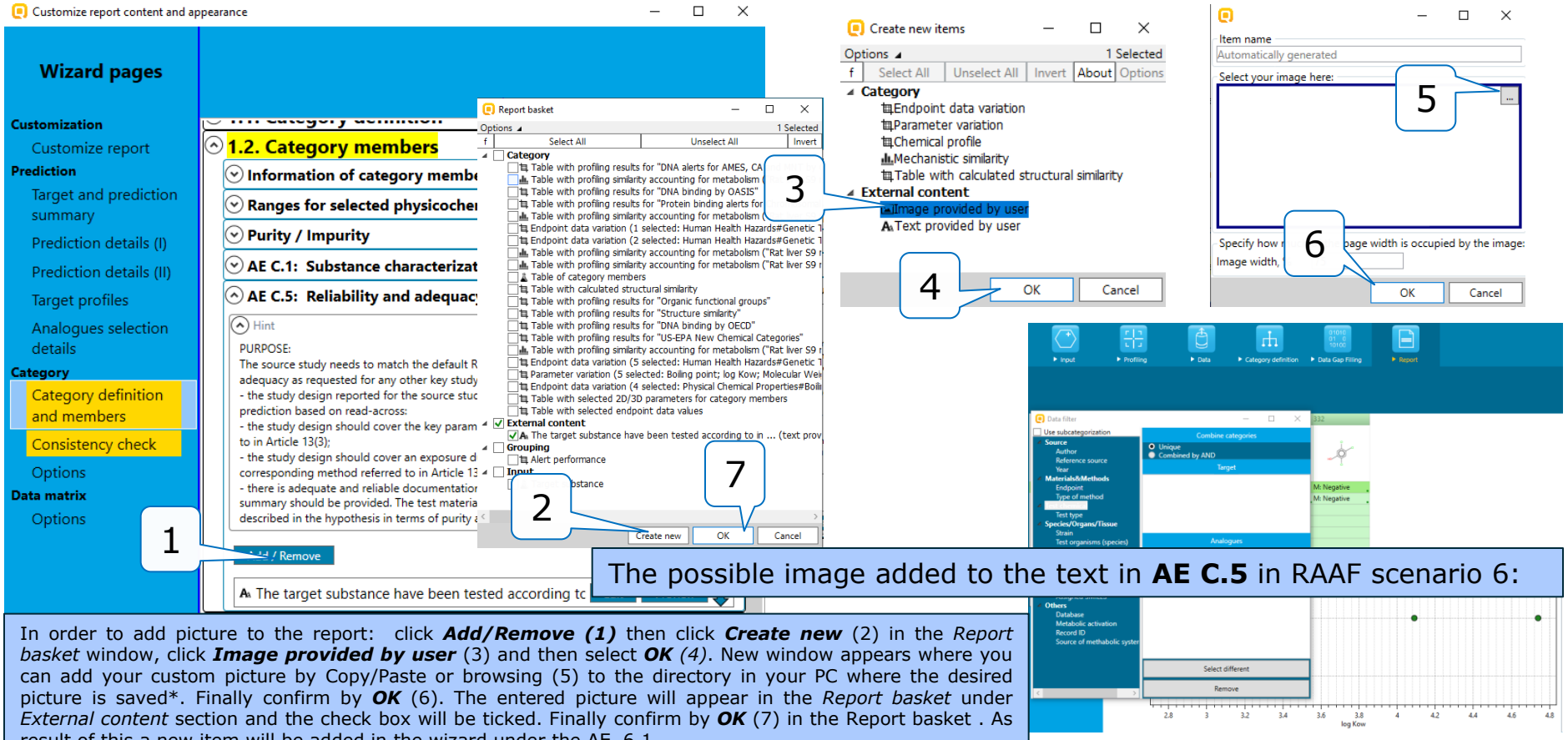
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Category definition and members*

AE C.5: *Reliability and adequacy of the source study(ies)*



The possible image added to the text in **AE C.5** in RAAF scenario 6:

In order to add picture to the report: click ***Add/Remove (1)*** then click ***Create new*** (2) in the *Report basket* window, click ***Image provided by user*** (3) and then select ***OK*** *(4)*. New window appears where you can add your custom picture by Copy/Paste or browsing (5) to the directory in your PC where the desired picture is saved*. Finally confirm by ***OK*** (6). The entered picture will appear in the *Report basket* under *External content* section and the check box will be ticked. Finally confirm by ***OK*** (7) in the Report basket . As result of this a new item will be added in the wizard under the AE 6.1.

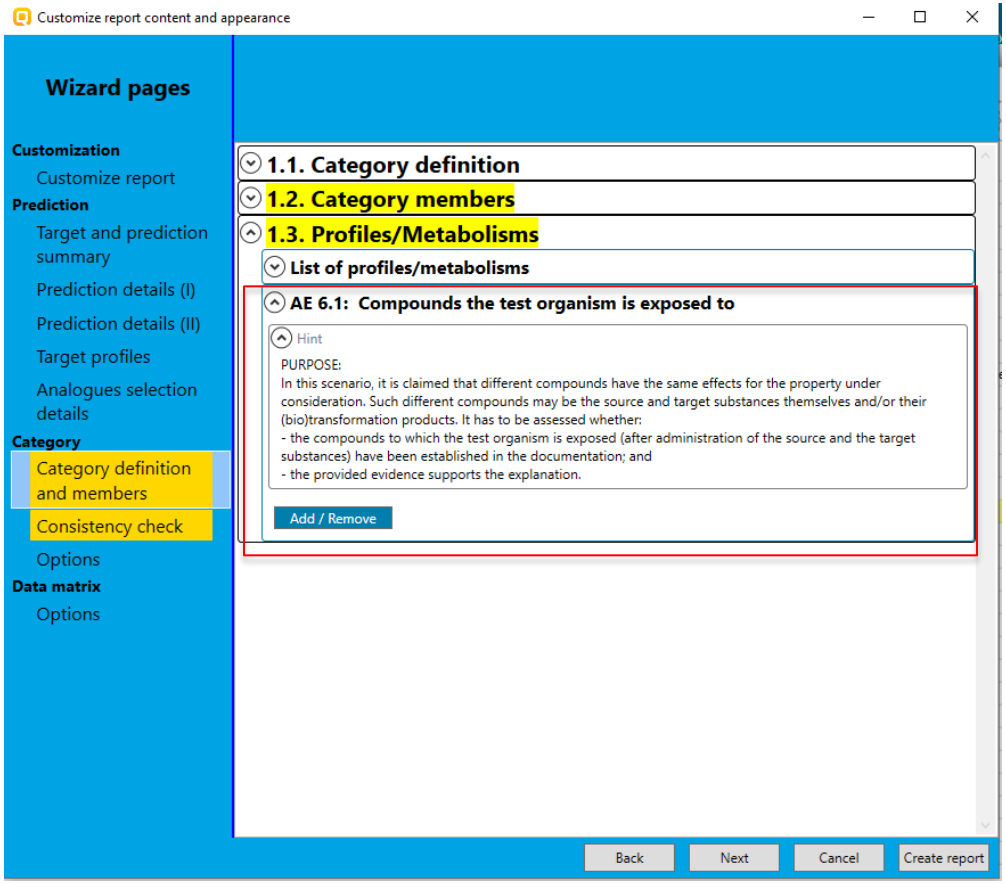
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Category definition and members*

AE 6.1: *Compounds the test organism is exposed to*



The possible example text that could be added to the **AE 6.1** is:

- Four source substances (B, C, D and E) are used to predict the property under consideration for Target A;
- Source substances (analogues) B, C, D and E have common reactivity pattern;
- Functionality that may cause chromosomal aberration by three different mechanisms for DNA interaction is identified in the metabolites of all category members;
- The primary group is defined based on "Quinones and Trihydroxybenzenes" accounting for *in vitro* Rat metabolism.

How to add the report item with external content is illustrated on the next slide:

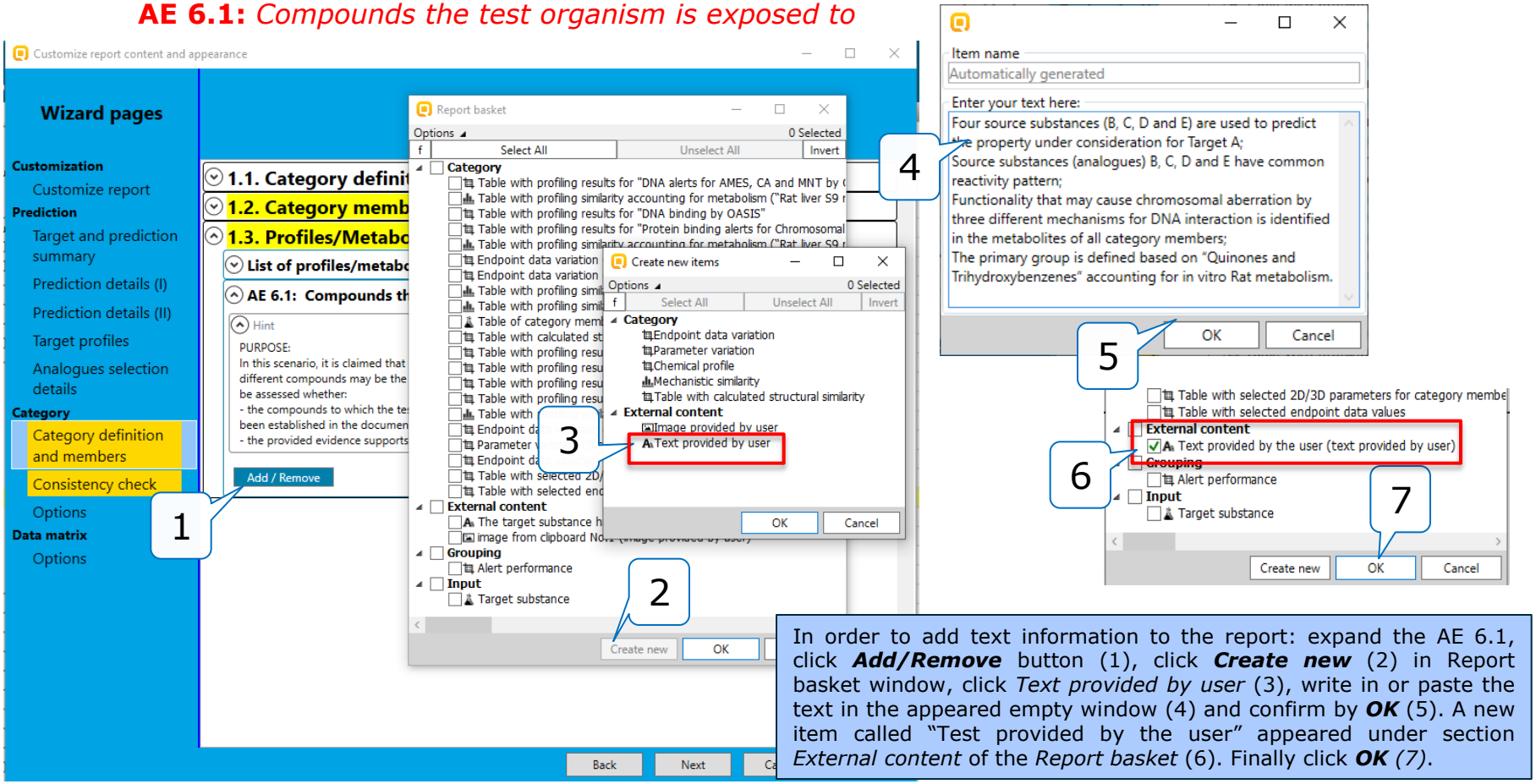
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Category definition and members*

AE 6.1: *Compounds the test organism is exposed to*



In order to add text information to the report: expand the AE 6.1, click ***Add/Remove*** button (1), click ***Create new*** (2) in Report basket window, click *Text provided by user* (3), write in or paste the text in the appeared empty window (4) and confirm by ***OK*** (5). A new item called "Test provided by the user" appeared under section *External content* of the *Report basket* (6). Finally click ***OK*** *(7)*.

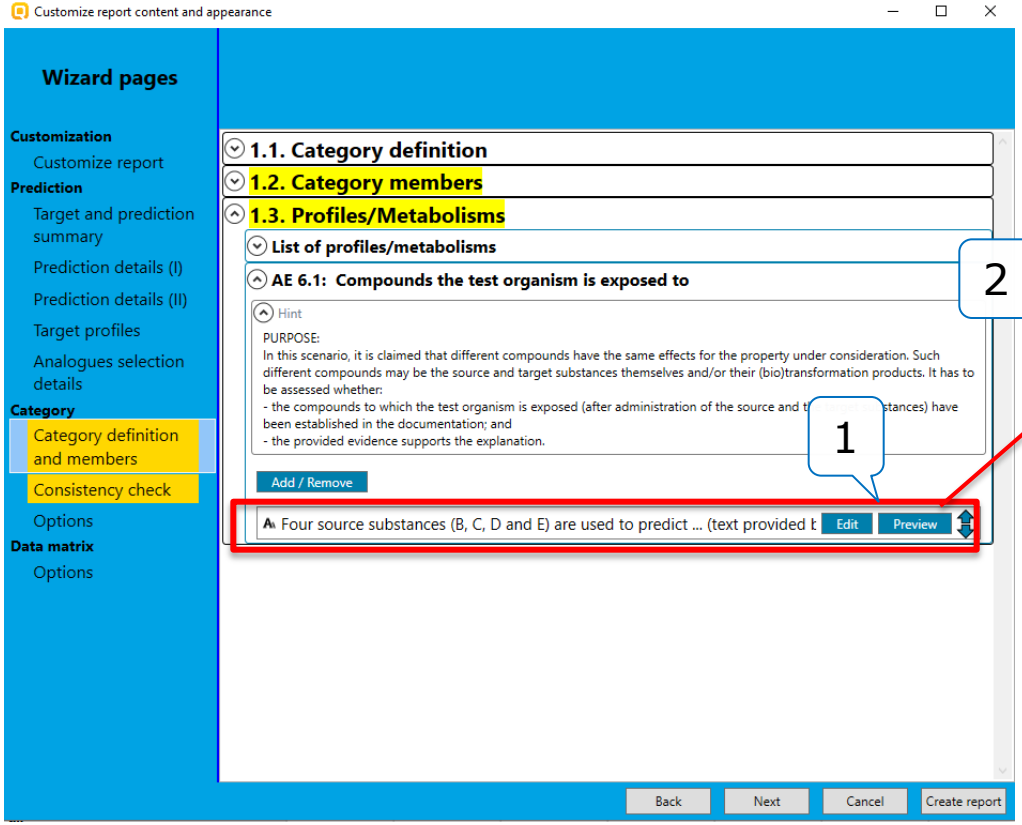
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Category definition and members*

AE 6.1: *Compounds the test organism is exposed to*



Example of how the example text will look in the generated report is shown below:

2

Four source substances (B, C, D and E) are used to predict ... (text provided by user)

Four source substances (B, C, D and E) are used to predict the property under consideration for Target A;

Source substances (analogues) B, C, D and E have common reactivity pattern;

Functionality that may cause chromosomal aberration by three different mechanisms for DNA interaction is identified in the metabolites of all category members;

The primary group is defined based on "Quinones and Trihydroxybenzenes" accounting for in vitro Rat metabolism.

There are two options (1) for editing or preview the generated report item. How will look the text item in the report is shown on the right (2)

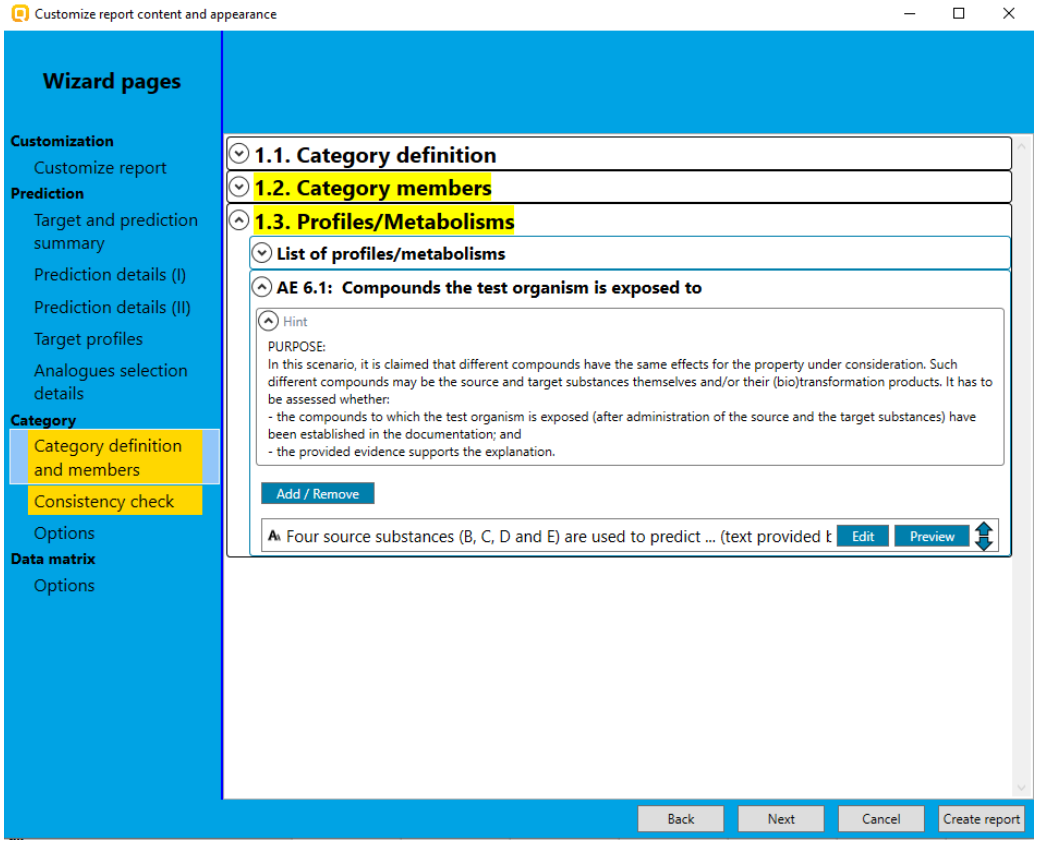
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Category definition and members*

AE 6.1: *Compounds the test organism is exposed to*



The possible image that could be added to the **AE 6.1** is:

Target A	Source B	Source C	Source D	Source E
				
EC Number:2097...	EC Number:2024...	EC Number:2132...	EC Number:2004...	EC Number:2017.
591-35-5	95-95-4	933-75-5	58-90-2	87-86-5
High	High	High	High	High
"3,5-dichlorophe...	"2,4,5-trichlorop...	"2,3,6-trichlorop...	2,3,4,6-tetrachlor...	"pentachlorophe.

How to add the image to the report is illustrated on the next slides:

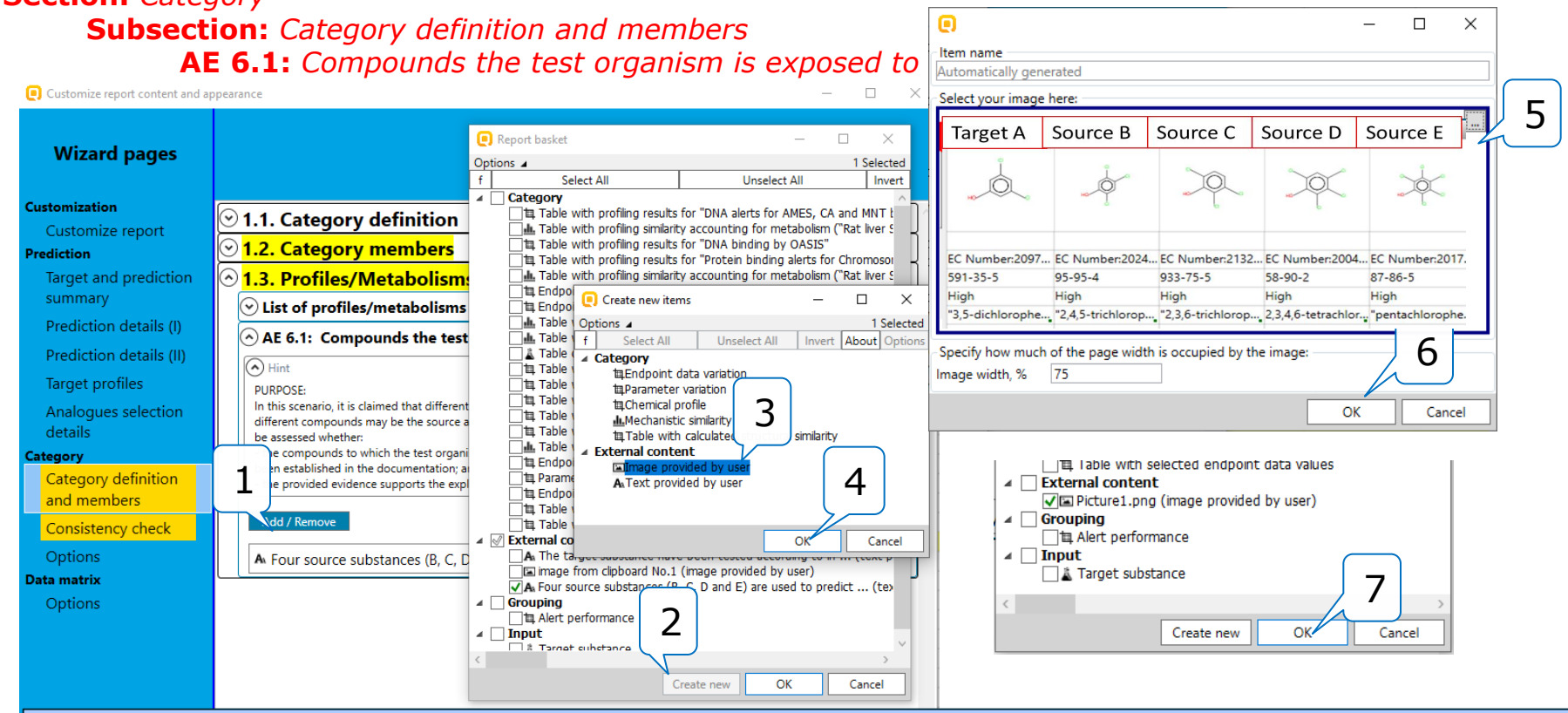
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Category definition and members*

AE 6.1: *Compounds the test organism is exposed to*



In order to add picture to the report: click ***Add/Remove (1)*** then click ***Create new*** (2) in the *Report basket* window, click ***Image provided by user*** (3) and then select ***OK*** *(4)*. New window appears where you can add your custom picture by Copy/Paste or browsing (5) to the directory in your PC where the desired picture is saved*. Finally confirm by ***OK*** (6). The entered picture will appear in the *Report basket* under *External content* section and the check box will be ticked. Finally confirm by ***OK*** (7) in the Report basket . As result of this a new item will be added in the wizard under the AE 6.1.

*In the current example a picture illustrating the target chemical marked as **Target A** and source substances (marked with Source from B to F) was prepared in advance.

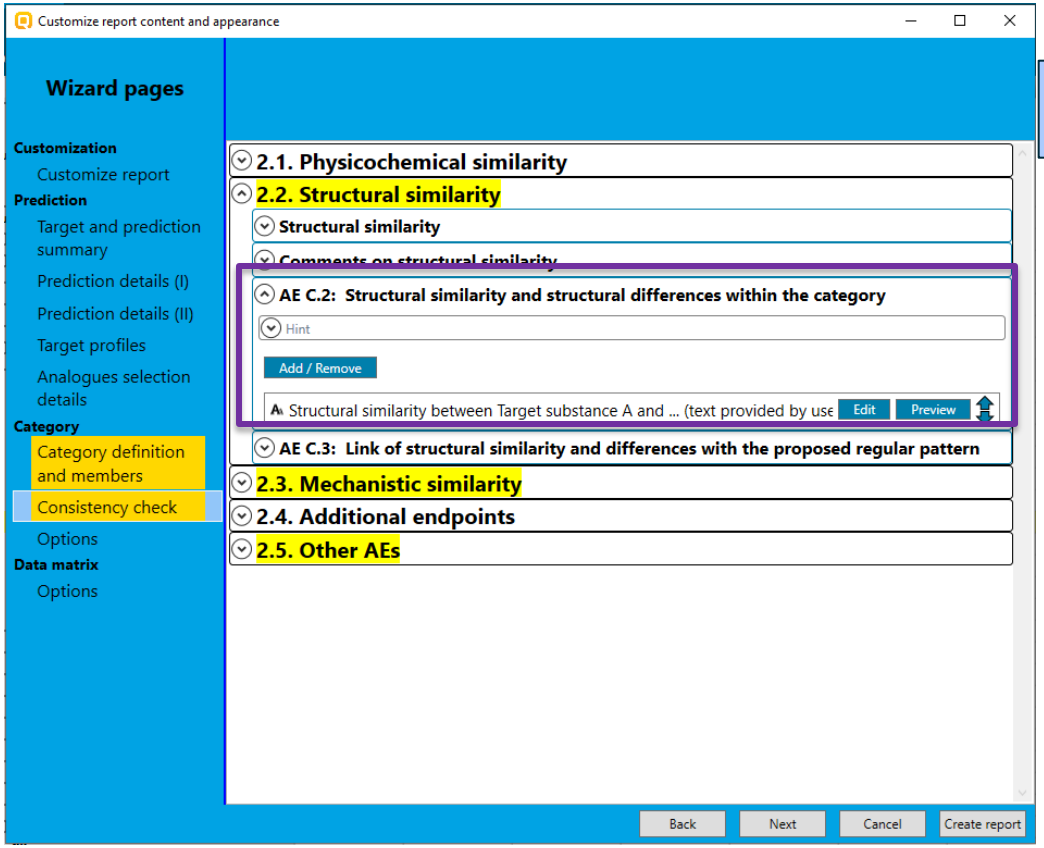
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Consistency check*

AE C.2: *Structural similarity and structural differences within the category*



The possible example text could be added to the **AE C.2** is:

- Structural similarity between Target substance A and five source substances (B, C, D and E) according to Str. similarity profiler is in the range of [28.6 – 76.2%]
- Target A and substances D, E have same functionalities with respect to OFG profiler
- Source substance B and C have same functionalities as target A, with exception of one group: *Aromatic perhalogencarbons*

Appendix with similarity table and profile statistics for OFG profiler could be provided here (see next two slides):

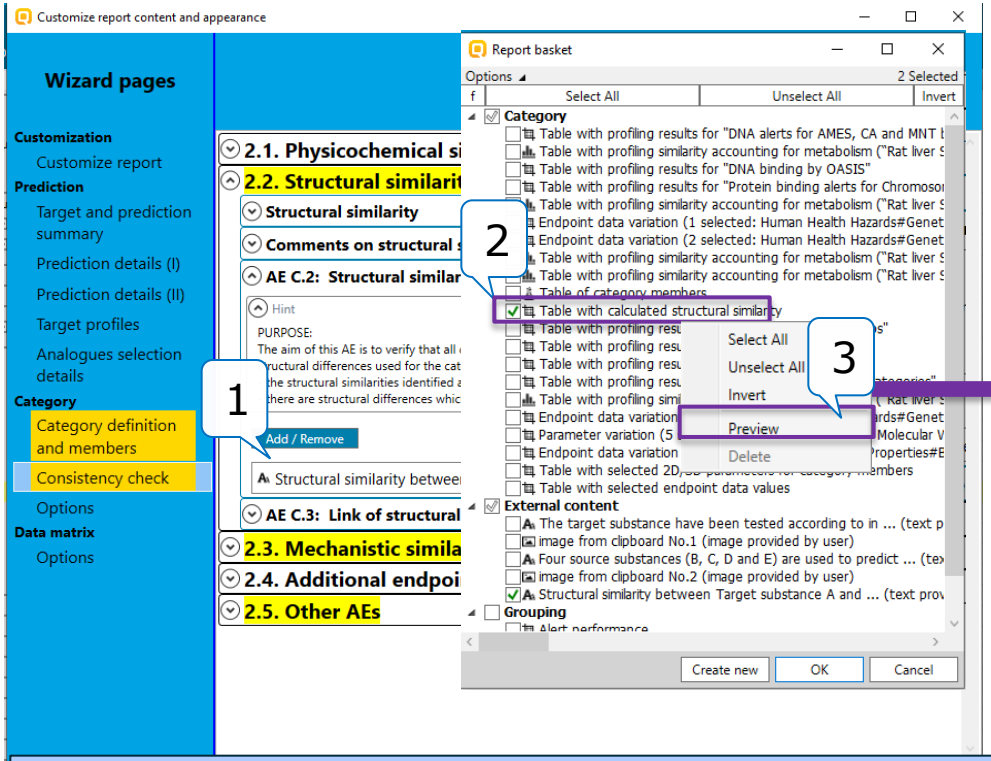
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Consistency check*

AE C.2: *Structural similarity and structural differences within the category*



Example with how the AE C.2 will look in the generated report is shown below:

4

Table with calculated structural similarity

Options

Mode: Hologram, CombineAllFeatures

Measure: Dice

Molecular features: AtomCenteredFragments

Atom characteristics: AtomType, CountHAttached, Hybridization

Calculated structure similarity

	1 CAS 591-35-5	327 CAS 87-86-5	328 CAS 58-90-2	330 CAS 933-75-5	332 CAS 95-95-4
1 CAS 591-35-5	100%	28.6 %	40 %	31.6 %	52.6 %
327 CAS 87-86-5	28.6 %	100%	69.6 %	54.5 %	36.4 %
328 CAS 58-90-2	40 %	69.6 %	100%	76.2 %	66.7 %
330 CAS 933-75-5	31.6 %	54.5 %	76.2 %	100%	60 %
332 CAS 95-95-4	52.6 %	36.4 %	66.7 %	60 %	100%

Two additional items have to be added in order to support the textual information:
Click ***Add/Remove*** button (1) and check the ***Table with calculated structural similarity*** item (2) which is stored in the *Report basket*. Right click and ***Preview*** the item (3). A table providing structural similarity between each of the category members is shown (4).

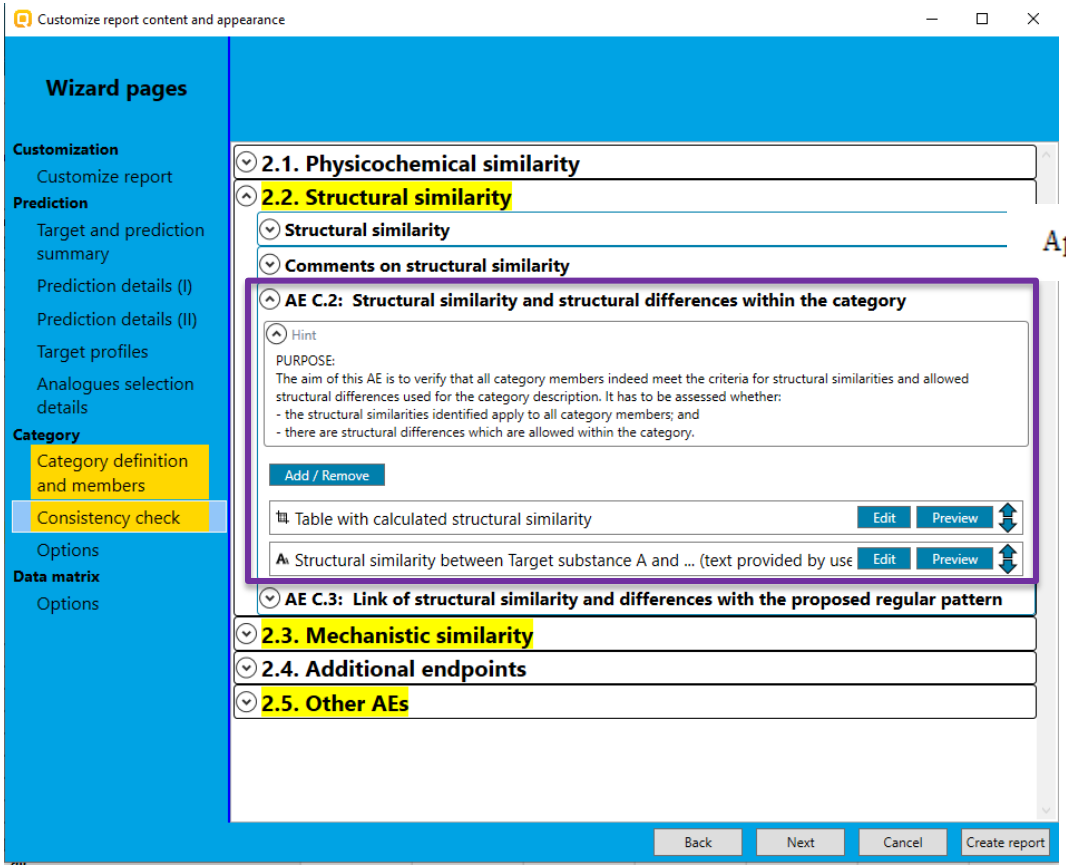
Report

Report generation according to RAAF Scenario 6

Section: *Category*

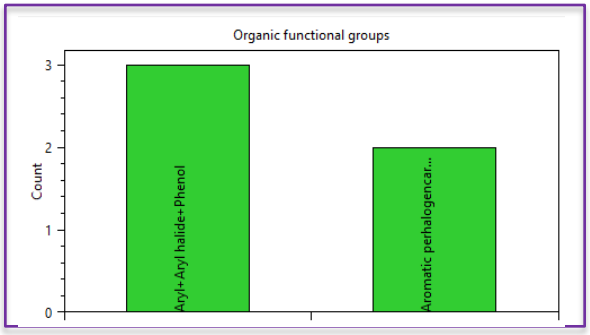
Subsection: *Consistency check*

AE C.2: *Structural similarity and structural differences within the category*



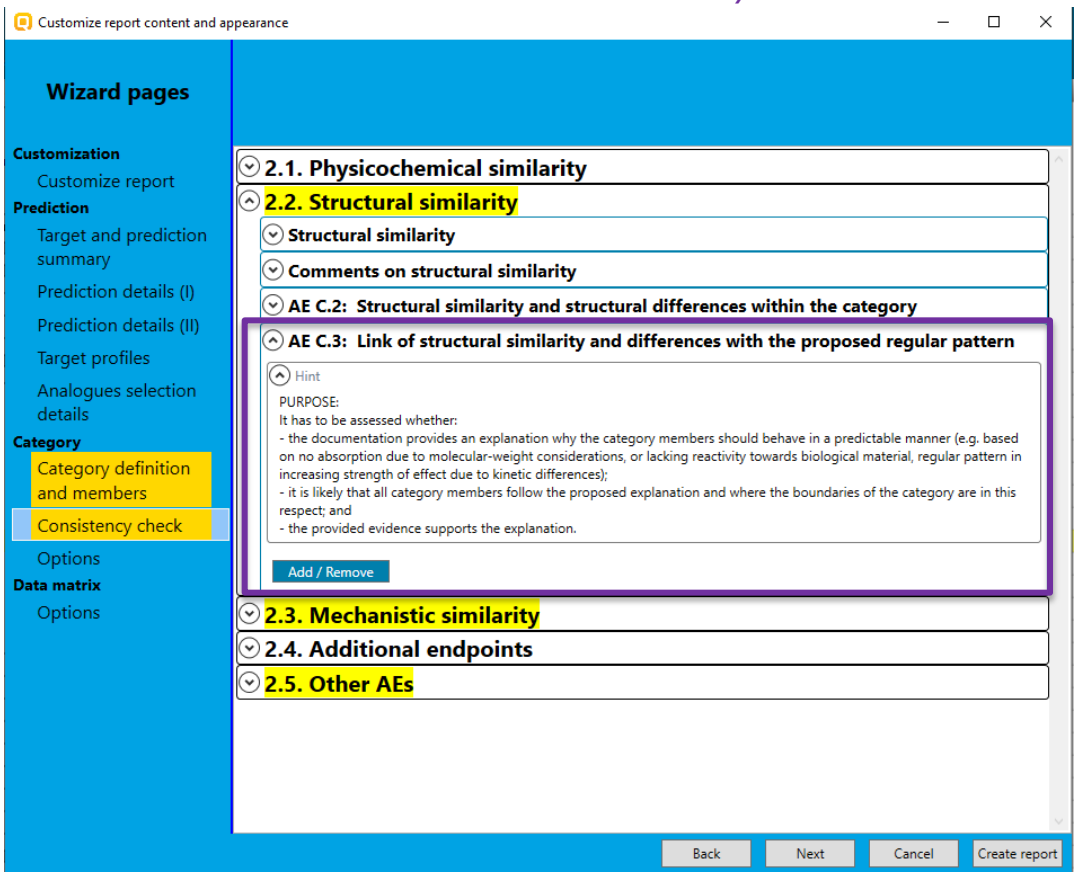
The possible report item containing image file could be added to the **AE C.2** (see slide 59 with instructions how to create it):

Appendix with profiling statistics based on OFG profiler could be added:



Report
Report generation according to RAAF Scenario 6

Section: *Category*
Subsection: *Consistency check*
AE C.3: *Link of structural similarity and differences with the proposed regular pattern*



The possible content of text added to the **AE C.3** is:

- No alerts related to chromosomal aberration have been identified in the structures of the target and the source substances as parents.
- Target and analogues are activated as a result of in vitro S9 metabolism simulator by generating "Quinones and Trihydroxybenzenes";
- In this respect, the structurally defined category from target (A) and four source substances (B, C, D, E) have common reactivity pattern of generated *in vitro* S9 metabolites.

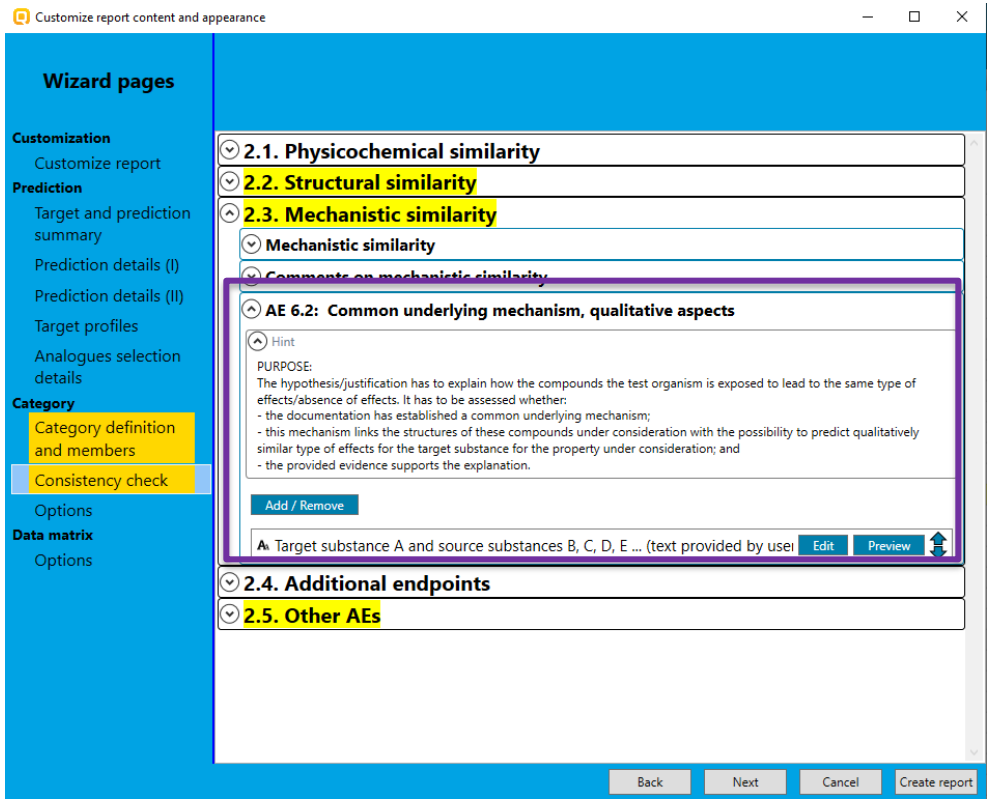
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Consistency check*

AE 6.2: *Common underlying mechanism, qualitative aspects*



The possible example text for **AE 6.2** is

- Target substance A and source substances B, C, D, E react via a common underling mechanism according to *DNA binding by OASIS*
- *Quinones and Trihydroxybenzenes* functionality alert is identified in all category members after metabolic activation (see Appendix Metabolite/Profiling)
- Common mechanism is illustrated in Appendix Metabolites/Profiling
- Our assumption is that the toxic effect is based on *Quinones and Trihydroxybenzenes* functionality
- As a primary group is used the *Quinones and Trihydroxybenzenes* group presented with three different mechanism of actions, supported by the calculated alert performance
- The similarity with respect to the metabolic pattern could be seen in **AE 4.5**. above.

Additionally, metabolic maps (for each of the analogues), produced by external software or found in the literature, could be included to this AE in order to support the mechanistic similarity of the category.

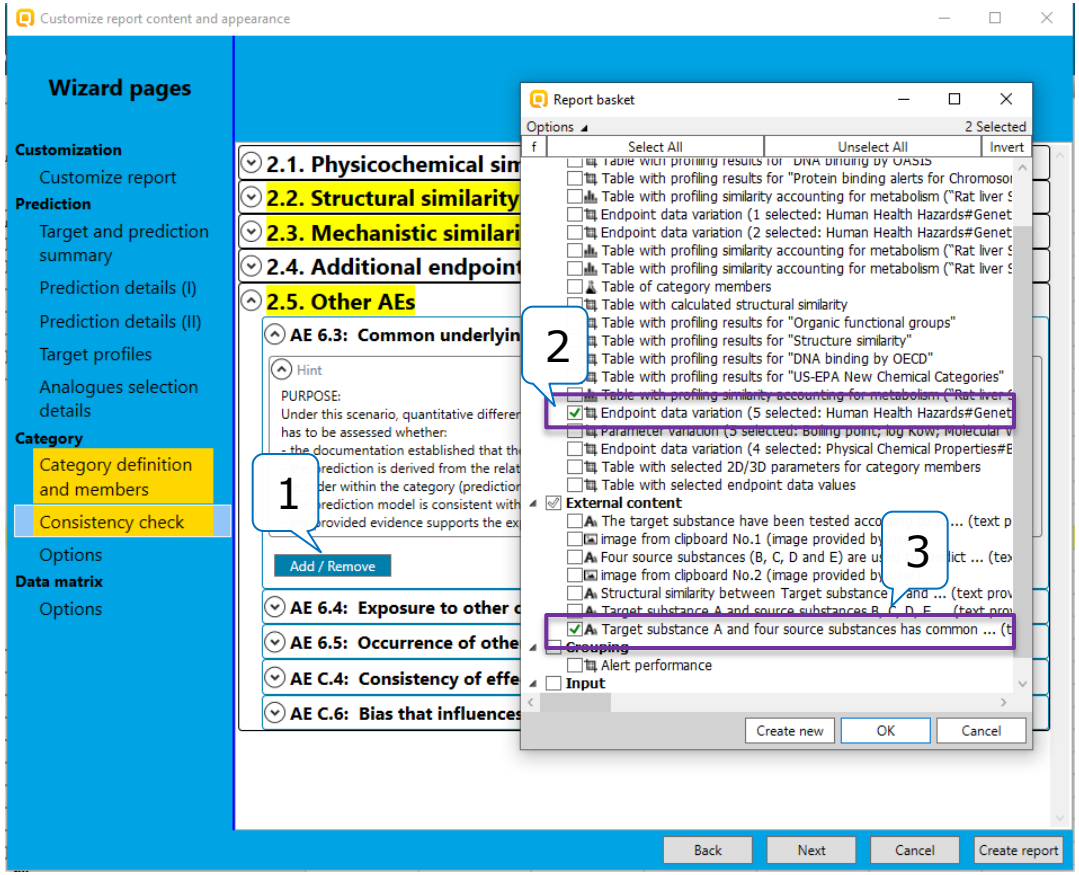
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Consistency check*

AE 6.3: *Common underlying mechanism, qualitative aspects*



The possible text added for the **AE 6.3** is:

- Target substance A and four source substances has common reactivity pattern
- They all formed *Quinones and Trihydroxybenzenes* functionality as metabolites responsible for the toxicity effects
- Toxic effects of all source substances and target are supported by the identified additional genotoxicity data – see *Data matrix* file generated by prediction Report

Include the ***Endpoint data variation*** item stored in the report basket (2).

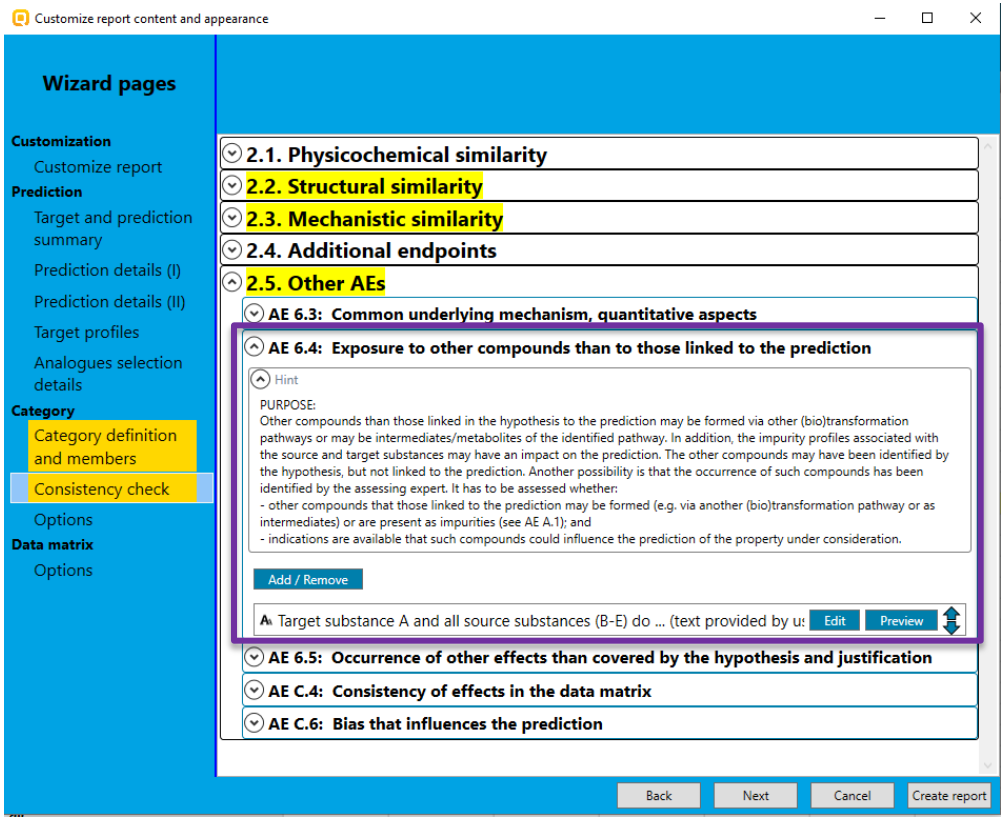
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Consistency check*

AE 6.4: *Exposure to other compounds than to those linked to the prediction*



The possible example text to added to the **AE 6.4** is:

- Target substance A and all source substances (B-E) do not have DNA alerts based on the endpoint-specific profiler and are not responsible for the toxicity effects acting as parents.
- Alerts for DNA binding causing chromosomal aberration (Quinones and Trihydroxybenzenes alert) are identified in the metabolites of the target and the source substances after *in vitro* Rat liver S9 activation
- Our assumption is that the toxicity effect of the category members is caused due to formation of active metabolites rather than of the chemicals themselves.

How to add the text is shown on slide 56.

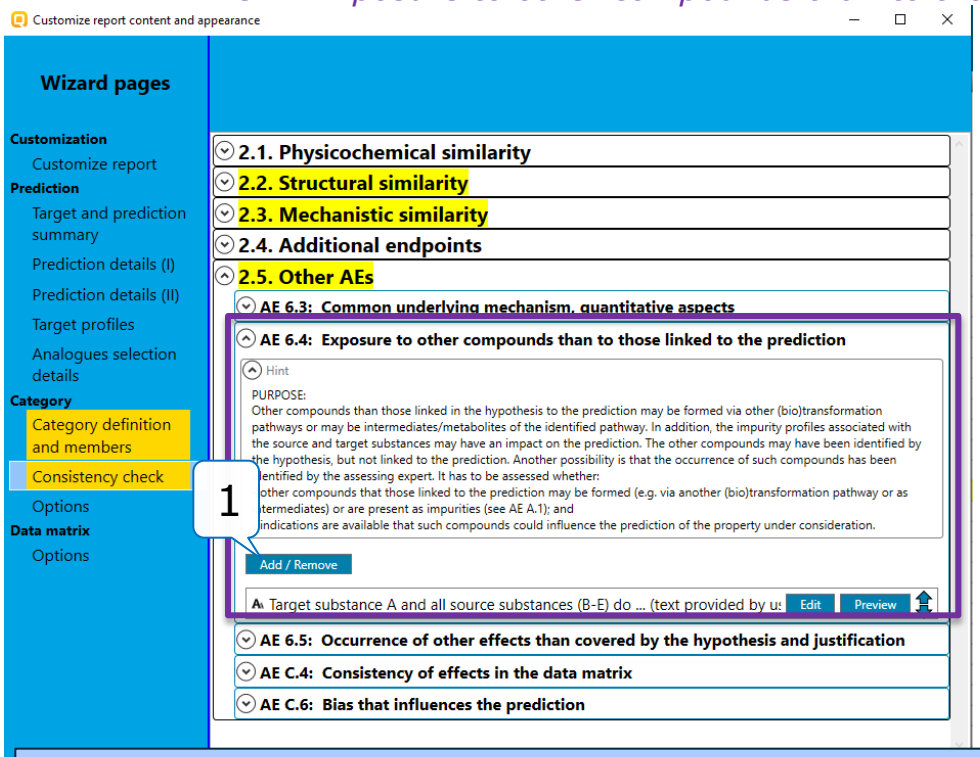
Report

Report generation according to RAAF Scenario 6

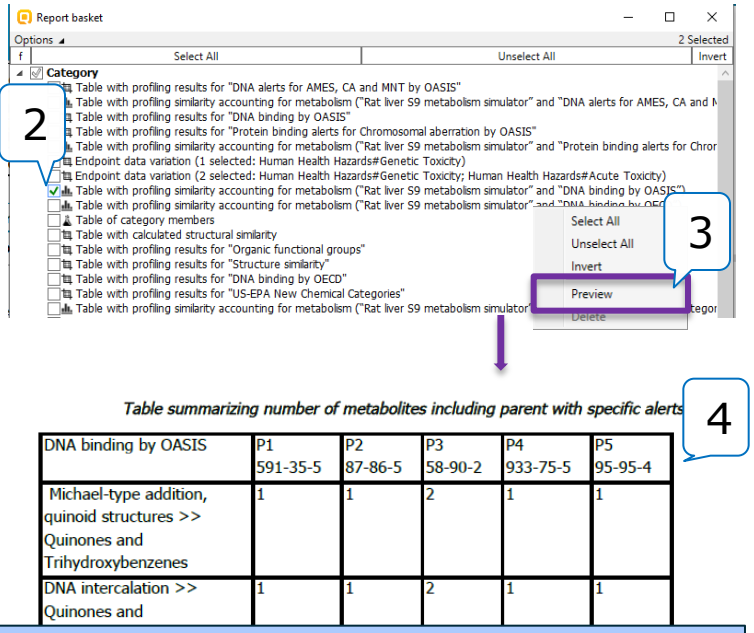
Section: *Category*

Subsection: *Consistency check*

AE 6.4: *Exposure to other compounds than to those linked to the prediction*



Example on how the **AE 6.4** will look in the generated report is shown:



DNA binding by OASIS	P1 591-35-5	P2 87-86-5	P3 58-90-2	P4 933-75-5	P5 95-95-4
Michael-type addition, quinoid structures >> Quinones and Trihydroxybenzenes	1	1	2	1	1
DNA intercalation >> Quinones and	1	1	2	1	1

Additionally to the text, the profiling similarity accounting for metabolism could be also included. To do this click on **Add/Remove** (1) button then check the box of ***Table with profiling similarity accounting for metabolism ….*** (2). The item is stored in the report basket, during the actions performed in the section Profiling. Right click and select ***Preview*** button (3). Tables summarizing the number of metabolite including the parent with the alerts is provided (4).

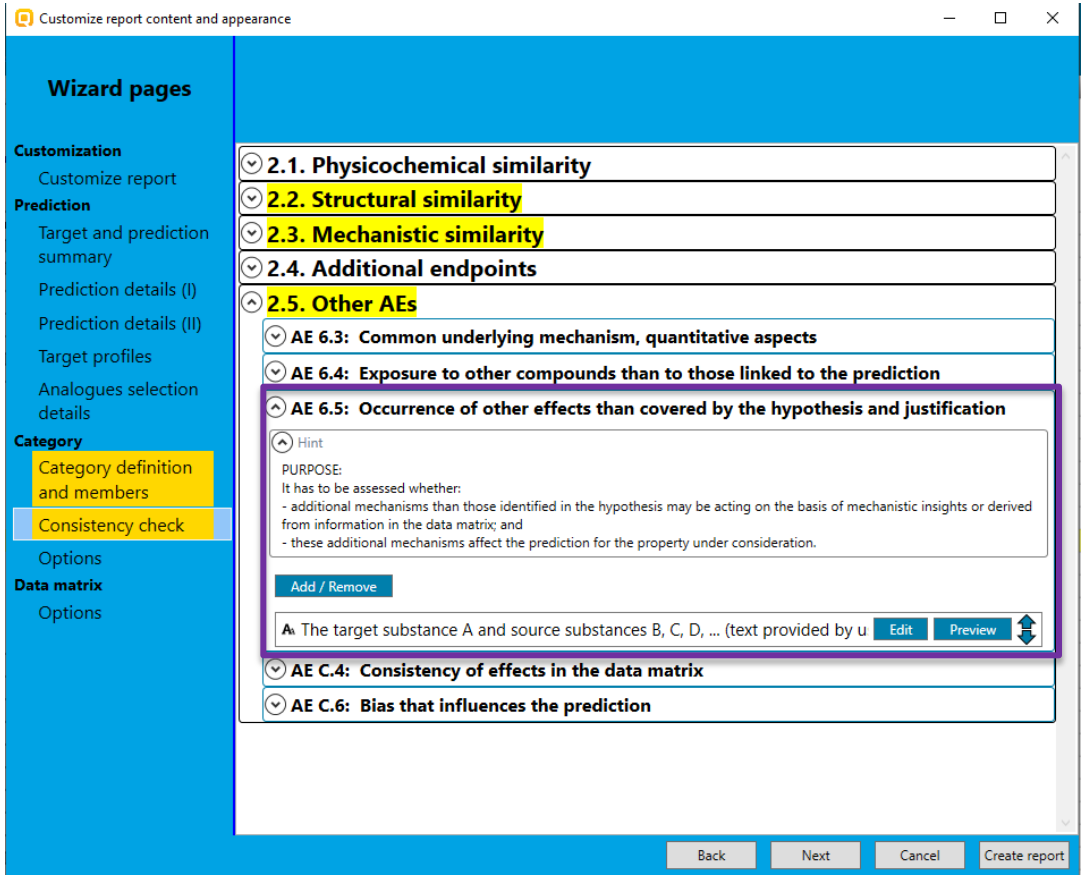
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Consistency check*

AE 6.5: *Occurrence of other effects than covered by the hypothesis and justification*



The possible example text to be added to the **AE 6.5** is:

- The target substance A and source substances B, C, D, E have common reactivity pattern based on presence of Quinones and Trihydroxybenzenes functionality in the structures of their metabolites;
- The Quinones and Trihydroxybenzenes functionality could cause toxicity effect by three different mechanisms for DNA binding;
- No other functionalities causing chromosomal aberration by DNA damage have been identified in the structures of the parents and metabolites.

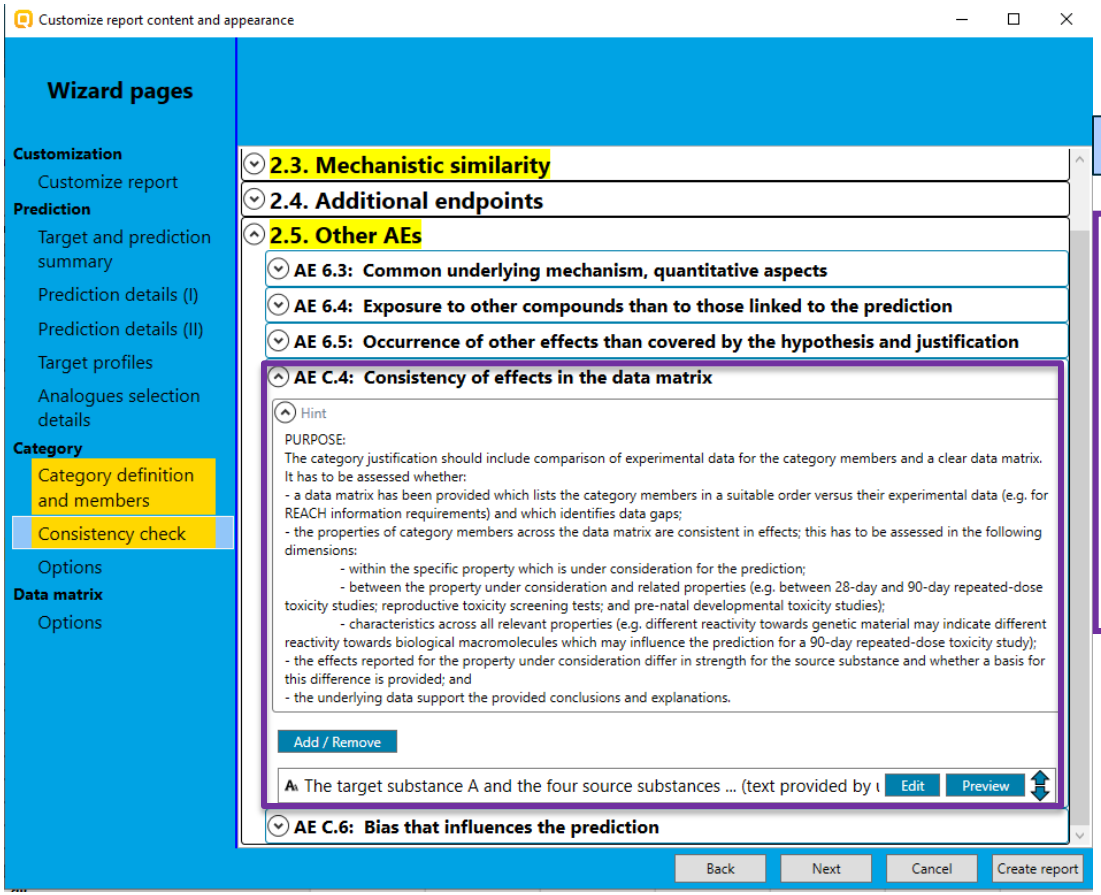
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Consistency check*

AE C.4: *Consistency of effects in the data matrix*



The possible text to added to the **AE C.4** is:

- The target substance A and the four source substances (B, C, D, E) show indication for chromosomal aberration effect.
- The latter is supported by the experimental data identified for CA effect (caused also by DNA damage) found for target and source substances.

Here could be provided also the data matrix snapshot or reference to the *Data matrix report* (see next slide).

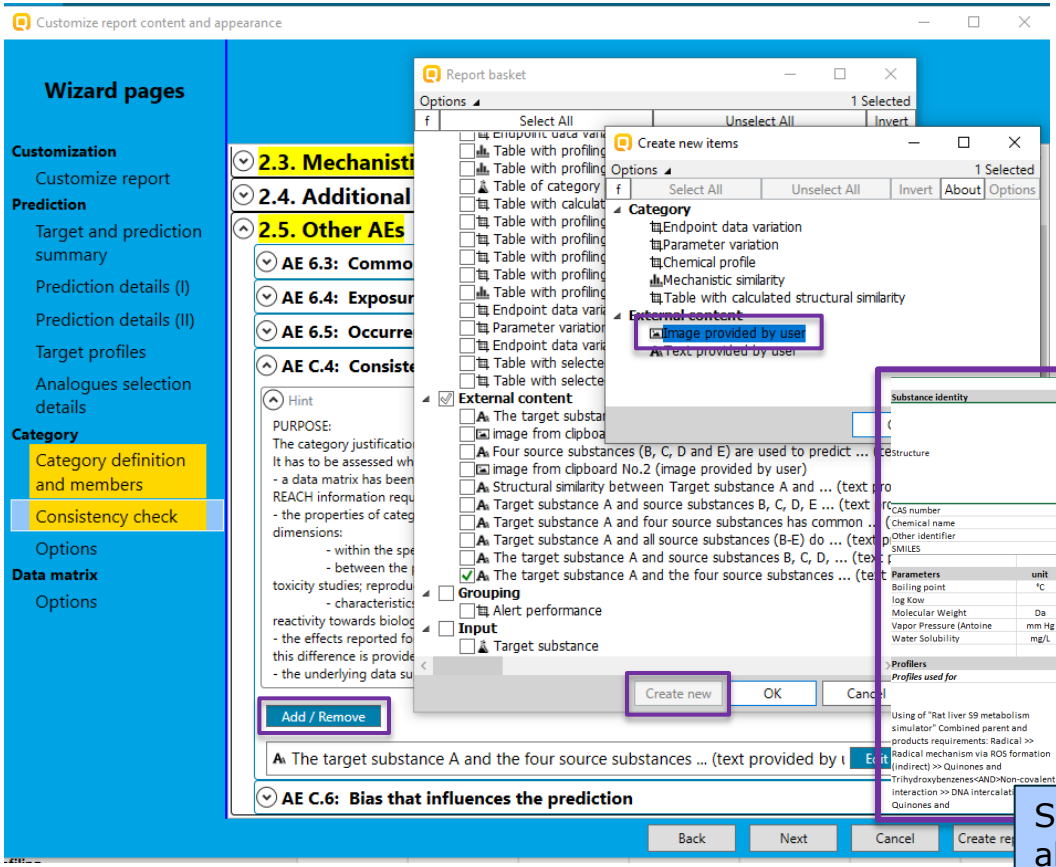
Report

Report generation according to RAAF Scenario 6

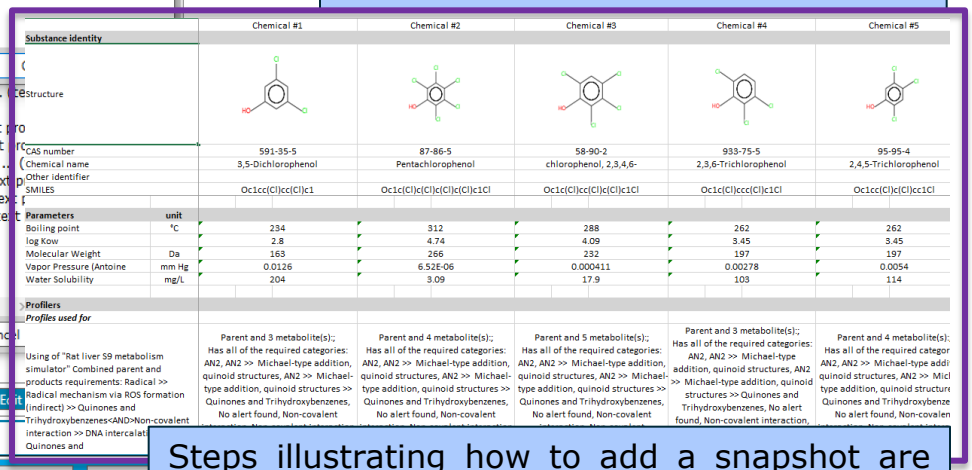
Section: *Category*

Subsection: *Consistency check*

AE C.4: *Consistency of effects in the data matrix*



The image added to the **AE C.4**:



Steps illustrating how to add a snapshot are already shown on slide 59.

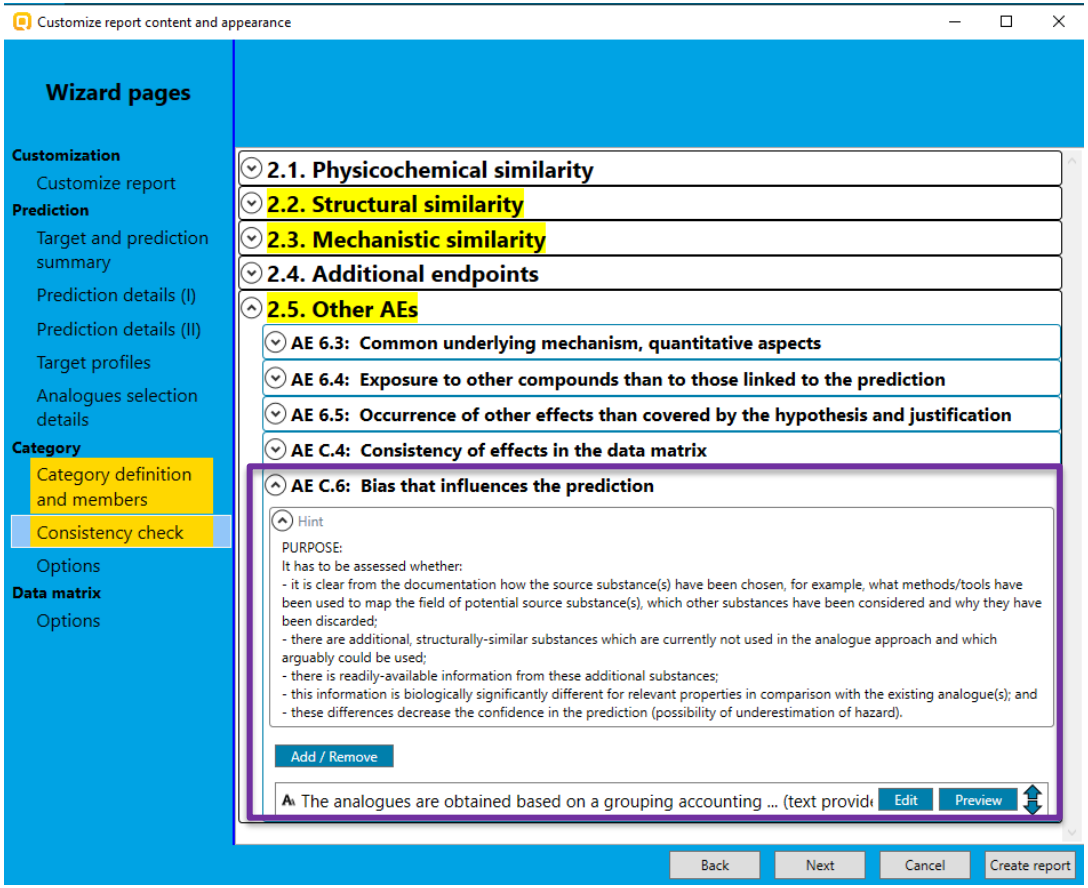
Report

Report generation according to RAAF Scenario 6

Section: *Category*

Subsection: *Consistency check*

AE C.6: *Bias that influences the prediction*



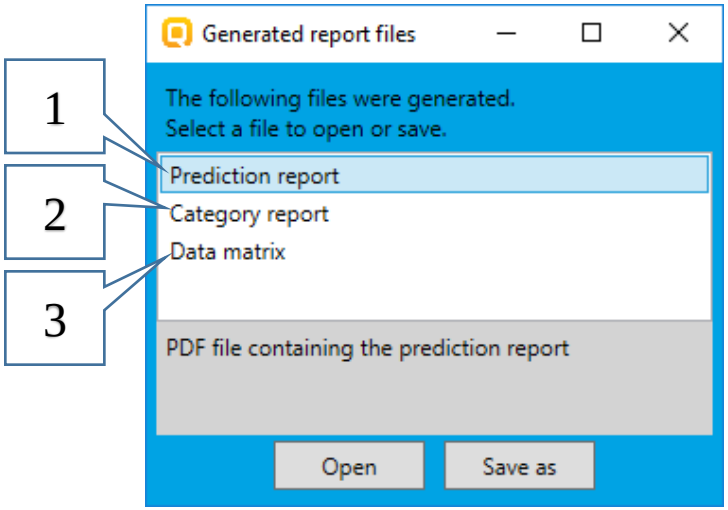
The possible example text which could be added to the **AE C.6** is:

- The analogues are obtained based on a grouping accounting for *in vitro* rat liver metabolism;
- All analogues having different metabolic pattern with respect to DNA interaction causing chromosomal aberration have been removed during the subcategorization process.
- The identified four analogues used in the read-across prediction have the common functional groups according to the OFG profiling scheme and the same reactivity pattern with respect to DNA interaction;

Report
Generation of report

After clicking on the *Create report* button, *Generated report files* window appears. It contains three types of files:

1. **Prediction report** - a PDF file containing the prediction information related to the target.
2. **Category report** - a PDF file containing information for the consistency of the final category (target plus used analogues)
3. **Data matrix** - a MS Excel file containing chemicals used for prediction along with their data for selected parameters, profiles and endpoint tree positions.



RAAF AEs are included in the second file.
All generated files should be provided when submitting a prediction.

Report
Generated report files

Prediction report

QSAR Toolbox prediction for single chemical

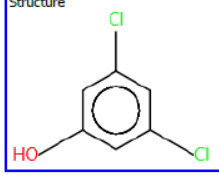
(In accordance with RAAF scenario 6)

Date: 14 Apr 2020
Author(s):
Contact details:

Target information

Structural information	Numerical identifiers	Chemical names
SMILES: Oc1cc(Cl)cc(Cl)c1	CAS#: 591-35-5 Other: EC Number:2097149	"3,5-dichlorophenol; dichlorophenol, 3,5- ;phenol, 3,5-dichlor o-;3,5-dichloro-phen ol" 3,5-dichloro-phenol 3,5-Dichlorophenol

Structure



Prediction

Predicted endpoint: Chromosome aberration; No effe
specified; No guideline specified
Predicted value: Positive
Unit/scale: Chromosome aberration I (Oasis)
Data gap filling method: Read-across analysis
Summary: *manually editable field*
Not provided by the user

Data matrix report

Substance identity		Chemical #1	Chemical #2	Chemical #3	Chemical #4	Chemical #5
Structure						
CAS number		591-35-5	87-86-5	58-90-2	933-75-5	95-95-4
Chemical name		3,5-Dichlorophenol	Pentachlorophenol	chlorophenol, 2,3,4,6-	2,3,6-Trichlorophenol	2,4,5-Trichlorophenol
Other identifier						
SMILES		Oc1cc(Cl)cc(Cl)c1	Oc1c(Cl)c(Cl)c(Cl)c(Cl)c1Cl	Oc1c(Cl)cc(Cl)c(Cl)c1Cl	Oc1c(Cl)ccc(Cl)c1Cl	Oc1cc(Cl)c(Cl)cc1Cl
Parameters	**unit**					
Boiling point	°C	234	312	288	262	262
log Kow		2.8	4.74	4.09	3.45	3.45
Molecular Weight	Da	163	266	232	197	197
Vapor Pressure (Antoine	mm Hg	0.0126	6.52E-06	0.000411	0.00278	0.0054
Water Solubility	mg/L	204	3.09	17.9	103	114
Profilers						
Profiles used for						
Using of "Rat liver S9 metabolism simulator" Combined parent and products requirements: Radical >> Radical mechanism via ROS formation (indirect) >> Quinones and Trihydroxybenzenes<AND>Non-covalent interaction >> DNA intercalation >> Quinones and		Parent and 3 metabolite(s):; Has all of the required categories: AN2, AN2 >> Michael-type addition, quinoid structures, AN2 >> Michael-type addition, quinoid structures >> Quinones and Trihydroxybenzenes, No alert found, Non-covalent interaction, Non-covalent interaction >> DNA intercalation, Non-covalent	Parent and 4 metabolite(s):; Has all of the required categories: AN2, AN2 >> Michael-type addition, quinoid structures, AN2 >> Michael-type addition, quinoid structures >> Quinones and Trihydroxybenzenes, No alert found, Non-covalent interaction, Non-covalent interaction >> DNA intercalation, Non-covalent	Parent and 5 metabolite(s):; Has all of the required categories: AN2, AN2 >> Michael-type addition, quinoid structures, AN2 >> Michael-type addition, quinoid structures >> Quinones and Trihydroxybenzenes, No alert found, Non-covalent interaction, Non-covalent interaction >> DNA intercalation,	Parent and 3 metabolite(s):; Has all of the required categories: AN2, AN2 >> Michael-type addition, quinoid structures, AN2 >> Michael-type addition, quinoid structures >> Quinones and Trihydroxybenzenes, No alert found, Non-covalent interaction, Non-covalent interaction >> DNA intercalation, Non-covalent	Parent and 4 metabolite(s): Has all of the required categor AN2, AN2 >> Michael-type addi quinoid structures, AN2 >> Micl type addition, quinoid structur Quinones and Trihydroxybenze No alert found, Non-covalen interaction, Non-covalent intera >> DNA intercalation, Non-cova

Category report

QSAR Toolbox report for category

(In accordance with RAAF scenario 6)

The selected RAAF scenario is specified in the first page

1. Category definition

1.1. Category definition

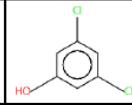
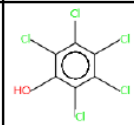
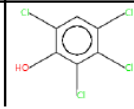
Category name *manually editable field*
Not provided by the user

Covered (target) endpoint(s)
- Human Health Hazards/Genetic Toxicity: Chromosome aberration, With S9, Chinese hamster, in Vitro Mammalian Chromosome Aberration Test, in Vitro

Category hypothesis *manually editable field*
Not provided by the user

1.2. Category members

Information of category members
Table of category members

#	CAS	Name	SMILES	Structure
		3,5-Dichlorophenol	Oc1cc(Cl)cc(Cl)c1	
		Pentachlorophenol	Oc1c(Cl)c(Cl)c(Cl)c(Cl)c1Cl	
		chlorophenol, 2,3,4,6-	Oc1c(Cl)cc(Cl)c(Cl)c1Cl	

Congratulations!

- You have now been introduced to the RAAF scenario;
- You have now been introduced to the *Report basket*.
- You have now been introduced to the AEs related to Scenario 6.
- Note, proficiency comes with practice!